IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of:

Susanne MATHEUS et al.   Examiner: KAUFMAN, CLAIRE M

Serial No.: 10/588,458   Group Art Unit: 1646

Filed: August 4, 2006   Confirmation No.: 5757

Title: **HIGHLY CONCENTRATED, LIQUID FORMULATIONS OF ANTI-EGFR ANTIBODIES**

BRIEF ON APPEAL UNDER 37 C.F.R. § 41.37

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Further to the Notice of Appeal filed June 19, 2009, attached is Appellants' Brief on Appeal, pursuant to 37 CFR §41.20(b)(2). An authorization to charge the requisite fee set forth under 37 CFR §41.20(b)(2) is also enclosed herewith.

This is an appeal from the decision of the Examiner finally rejecting claims 1, 4, 8, 11, 16, 17 and 21-24 of the above-identified application under 35 USC §103(a). The <u>final</u> rejection was mailed on March 19, 2009.

(I) REAL PARTY IN INTEREST

Merck Patent GmbH of Darmstadt, GERMANY is the Assignee of Record of the entire right, title, and interest in and to the above-identified application, as recorded in the U.S. Patent and Trademark Office on August 4, 2006, at Reel/Frame 018167 / 0734.

(II) RELATED APPEALS AND INTERFERENCES

Appellants, their legal representative and the assignee are not aware of any related appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the instant appeal.

(III) STATUS OF THE CLAIMS

Claims rejected: Claims 1, 4, 8, 11, 16, 17 and 21-24.

Claims allowed: (None).

Claims cancelled: Claims 2, 3, 5-7, 9, 10, and 12-15.

Claims withdrawn: Claims 18-20.

Claims on Appeal: Claims 1, 4, 8, 11, 16, 17 and 21-24. (Copy of claims on appeal in the attached Appendix).

(IV) STATUS OF AMENDMENTS

On May 19, 2009, Appellants timely filed a reply to the final Office Action of March 19, 2009 containing claim amendments (an amendment after-final under 37 CFR §1.114(a)). In an Advisory Action mailed June 11, 2009, the Examiner stated that the amendments would not be entered as they "would require further consideration as to proposed newly-listed ingredients' activity." Thus, the amendments presented with the non-final Reply of December 9, 2008 (to the Office Action of September 12, 2008) are entered and are reflected in the claims on appeal shown in the attached Appendix of Claims.

(V) SUMMARY OF CLAIMED SUBJECT MATTER

One aspect of Appellants' invention (independent claim 1) is directed to a process for the preparation of a highly concentrated, liquid formulation containing monoclonal antibody c225 (Mab c225) or monoclonal antibody h425 (Mab h425) at a concentration of 50 mg/ml to 180 mg/ml. The process includes ultrafiltrating the preparation containing said Mab c225 or Mab h425. See, for example, original claims 1, 3 and 7. See also, the disclosure contained in, for example, page 7, lines 10-25 of the originally-filed specification and the Examples section beginning at page 36 of the specification, as originally filed. Claim 4, which is dependent on claim 1, is directed to a process of preparing highly concentrated, liquid formulations containing c225 or h425, wherein the concentration of the antibody in the formulation is 100 – 150 mg/ml. Support for the claim can be found in, for example, the disclosure contained in page 7, lines 12-16 of the originally-filed specification.

In a related embodiment (independent claim 22), the present invention comprises methods for the preparation of a highly concentrated, liquid formulation containing monoclonal antibody c225 (Mab c225) or monoclonal antibody h425 (Mab h425) at a concentration of 50 mg/ml to 180 mg/ml, wherein the method consists essentially of ultrafiltrating a preparation comprising the Mab c225 or Mab h425. This claimed embodiment is supported, at least, by the disclosure contained in the Examples.

Another aspect of Appellants' invention (independent claim 8) is directed to a highly concentrated, liquid formulation containing monoclonal antibody c225 (Mab c225) or monoclonal antibody h425 (Mab h425), wherein the concentration of said antibody in said highly concentrated, liquid formulation thus prepared is 50 mg/ml to 180 mg/ml. See page 18, lines 13–17 of the originally-filed specification for support. Claims 11 and 17 are directly or indirectly dependent on the aforementioned independent claim 8, and recite additional aspects of the formulations of the instant invention. For example, claim 11 recites that the concentration of the antibody in said highly concentrated, liquid formulation is 100 – 150 mg/ml. See, for example, the disclosure contained in page 7, lines 12-16 of the originally-filed specification. Claim 17 recites that the formulation further comprises an excipient, adjuvant or an additional pharmaceutical active ingredient. Support for the claim can be found in, for example, page 11, lines 16-24. For a disclosure on types additional pharmaceutical active ingredients, see page 21, lines 12–17 of the originally-filed specification.

In another related aspect, the present invention (independent claim 23) relates to a highly concentrated, liquid formulation consisting essentially of monoclonal antibody c225 (Mab c225) or monoclonal antibody h425 (Mab h425), wherein the concentration of said antibody in said highly concentrated, liquid formulation is 50 mg/ml to 180 mg/ml. Claim 24, which is dependent on claim 23, is directed to antibody formulations consisting essentially of c225 or h425 at a concentration of 100 – 150 mg/ml. Support for this aspect can be found in, for example, the disclosure contained in Examples.

Yet another aspect of the instant invention relates to storage stable pharmaceutical preparations and kits comprising the highly concentrated, liquid formulations of c225 or h425 antibodies of the present invention. To this end, claim 16 is directed to a storage stable pharmaceutical preparation comprising a highly concentrated, liquid formulation of c225 or h425 antibody and an acceptable excipient or adjuvant. Support for the claim can be found in, at least, the disclosure contained in page 17, ¶2 and ¶3 of the originally-filed specification. Claim 21 is directed to a kit comprising a highly concentrated, liquid formulation of c225 or h425 antibody and an additional medicament active ingredient. The claim is supported by the disclosure contained in, for example, the paragraph bridging pages 22 and 23 of the originally-filed specification.

(VI) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL
Appellants request a review of the following remaining grounds of rejection. For

each ground, any separate grouping of the claims subject to that rejection is indicated. As per the requirements under 37 CFR §1.113(a), objections of formal nature are not being appealed.

(1)   The rejection of claims 1, 4, 8, 11, 16–17 and 21–24 are rejected under §103(a) as allegedly rendered obvious by Sridhar (*Lancet Oncology*, 2003) in view of Arvinte et al. (WO 02/96457).

<div align="center">Grouping of claims</div>

The claims stand or fall together with independent claims 1, 8, 22 and 23.

<div align="center">(VII) ARGUMENT</div>

Rejection under 35 U.S.C. §103(a)

Claims 1, 4, 8, 11, 16-17 and 21-24, on appeal, are not rendered obvious by the aforementioned Sridhar (*Lancet Oncology*, 2003) and Arvinte et al. (WO 02/96457). The rejection under 35 U.S.C. §103(a) is not supported on the record as a whole and should be reversed.

The basis for the obviousness rejection can be found in the paragraphs bridging pages 3 and 4 of the Office Action mailed March 19, 2009 (and pages 6 and 7 of the Office Action mailed September 9, 2008). Therein it is alleged that:

> "Sridhar et al. teaches (two paragraphs beginning p. 398 col. 2 with the second full paragraph that
>
>> Monoclonal antibodies have been developed that target different members of the EGFR superfamily. They are highly specific with few side-effects and may be synergistic with chemotherapy and radiation....
>> Cetuximab (IMC-C225) is a human-murine chimeric IgG monoclonal antibody that competitively binds to the extracellular domain of EGFR.... Preclinical studies show that cetuximab inhibits the proliferation of cell lines expressing EGFR and increases the cytotoxic activity of chemotherapy and radiation. Cetuximab alone and in combination with chemotherapy or radiotherapy was generally well tolerated in phase I trials.... Cetuximab in combination with chemotherapy has shown activity in head and neck and colorectal cancers with acceptable toxic effects.
>
> Sridhar et al. also discuss other EGFR antibodies in clinical trials as cancer therapies, including EMD72000 (Table 1 and p. 400, col. 1, 1st full paragraph. Sridhar et al. do not discuss antibody formula concentrations or the means of concentrating an antibody formulation."

The final Office Action at page 4 alleges that this aspect is rectified by Arvinte's teachings of concentrated antibody preparations (50 mg/ml to 250 mg/ml) and means of preparing such. It is further alleged at page 4 of the Office Action that Arvinte

additionally discloses "antibodies may be monoclonal, chimeric antibodies which are humanized, antibody fragments and antibody derivatives which are PEGylated." The Office Action then concludes that the instantly claimed highly concentrated, liquid antibody formulations and means of preparing such, for example, via ultracentrifugation, are rendered obvious by Sridhar in view of Arvinte. Appellants respectfully disagree with these contentions.

An aspect of the present invention is directed to the preparation of highly concentrated, liquid formulations of Mab c225 and Mab h425 as stable, ready-to-use solutions having low viscosity, low application volumes (for use as pharmaceutical preparations) and that are applicable for subcutaneous administration. Preparation of highly concentrated, liquid formulations of antibodies are afflicted with technical challenges and routine protocols for protein concentration are not always applicable for large proteins with specific properties, such as, monoclonal antibodies that are usable in the clinical setting. The methods of the present invention allow for the preparation of highly concentrated, liquid formulations of the aforementioned anti-EGFR antibodies, and the ready formulation thereof as pharmaceutical preparations and/or kits for therapeutic and diagnostic applications. Appellants further submit that for each individual antibody and especially for each monoclonal antibody a specific method has to be developed to arrive at a preparation of highly concentrated formulations. For example, it was art-recognized that the formulation of such proteins [possessing multiple functional groups in addition to complex three-dimensional structures] poses special problems. For a protein to remain biologically active, a formulation must preserve intact the conformational integrity of at least a core sequence of the protein's amino acids while at the same time protecting the protein's multiple functional groups from degradation." See, Lam's disclosure in the BACKGROUND section of US patent No. 6,171,586. To this end, it was also recognized that <u>monoclonal antibodies poses a difficult problem with respect to high concentration</u>, especially if pharmaceutically critical stabilizers should be omitted. As explicitly stated under MPEP §2145, "proceeding contrary to accepted wisdom in the art is evidence of non-obviousness. *In re Hedges,* 783 F.2d 1038, 228 USPQ 685 (Fed. Cir. 1986)." As such, the antibody preparations, compositions, kits of the present invention, including methods for obtaining such are inventive over the cited references. Favorable reconsideration is respectfully requested.

Arvinte in combination with Sridhar also does not render the claims obvious because nothing motivates a skilled worker to choose precisely Mab c225 or Mab h425 and combine it with precisely a generic process of concentrating proteins, especially

since the details and examples of the secondary reference would point particularly to methods of concentrating other types of antibody molecules. To this end, Example 1 of Arvinte discloses methods of preparing omalizumab (i.e., anti-IgE antibody E25) at a concentration of 99 mg/ml. This antibody is not equivalent to c225 or h425 monoclonal antibodies of the instant invention, which are characterized by specific epitope-binding capability. As disclosed in the background section of the specification, these antibodies *specifically* bind to EGFR with high binding-affinity. Example 2 in Arvinte is directed to methods for obtaining the aforementioned E25 antibody in a concentrated formulation of 214 mg/ml and 297mg/ml. Examples 3-5 provide biochemical analysis of concentrated formulations of E25. In Example 7, the reference discloses a general method for the preparation of high concentrated liquid formulations comprising E25. In all the Examples, Arvinte generically discloses formulations comprising antibodies that bind *to a very broad target*, for example, immunoglobulin E (IgE) molecules. There is no disclosure of how concentrated formulations of antibodies that bind to specific epitopes, such as anti-EGFR antibodies, may be prepared. More specifically, neither Sridhar nor Arvinte provides any hint or suggestion that the specific-epitope binding antibodies of the present invention (i.e., Mab c225 or Mab h425) can be prepared as highly concentrated formulations (50 mg/ml to 180 mg/ml). Without such motivation, there can be no obviousness. *In re Baird*, 16 F.2d 380 (Fed.Cir. 1994).

Withdrawal of the rejection is respectfully requested.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

/Sagun KC/

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Sagun KC, L0510
For Appellant(s)

/Anthony J. Zelano/

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Anthony J. Zelano, Reg. No. 27,969
Attorney for Appellant(s)

MILLEN, WHITE, ZELANO
  & BRANIGAN, P.C.
Arlington Courthouse Plaza 1, Suite 1400
2200 Clarendon Boulevard
Arlington, Virginia 22201
Telephone: (703) 243-6333
Facsimile: (703) 243-6410

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Date: August 19, 2009

(VIII) CLAIMS APPENDIX

Claim 1.   A process for the preparation of a highly concentrated, liquid formulation comprising monoclonal antibody c225 (Mab c225) or monoclonal antibody h425 (Mab h425), comprising ultrafiltrating a preparation comprising said Mab c225 or Mab h425, wherein the concentration of said antibody in said highly concentrated, liquid formulation thus prepared is 50 mg/ml to 180 mg/ml.

Claim 4.   The process according to Claim 1, wherein the concentration of said antibody in said highly concentrated, liquid formulation is 100 – 150 mg/ml.

Claim 8.   A highly concentrated, liquid formulation comprising monoclonal antibody c225 (Mab c225) or monoclonal antibody h425 (Mab h425), wherein the concentration of said antibody in said highly concentrated, liquid formulation thus prepared is 50 mg/ml to 180 mg/ml.

Claim 11.   The highly concentrated, liquid formulation according to Claim 8, wherein the concentration of said antibody is 100 – 150 mg/ml.

Claim 16.   A storage-stable pharmaceutical preparation which comprises a highly concentrated, liquid formulation according to claim 7 and an acceptable excipient or adjuvant.

Claim 17.   A highly concentrated, liquid formulation according to Claim 8 which optionally further comprises an excipient, adjuvant or an additional pharmaceutical active ingredient.

Claim 21.   A kit which comprises a highly concentrated, liquid formulation according to claim 7 and an additional medicament active ingredient.

Claim 22.   A process for the preparation of a highly concentrated, liquid formulation comprising monoclonal antibody c225 (Mab c225) or monoclonal antibody h425 (Mab h425) consisting essentially of ultrafiltrating a preparation comprising said Mab c225 or Mab h425, wherein the concentration of said antibody in said highly concentrated, liquid formulation thus prepared is 50 mg/ml to 180 mg/ml.

Claim 23.   A highly concentrated, liquid formulation consisting essentially of

monoclonal antibody c225 (Mab c225) or monoclonal antibody h425 (Mab h425), wherein the concentration of said antibody in said highly concentrated, liquid formulation is 50 mg/ml to 180 mg/ml.

Claim 24. The highly concentrated, liquid formulation according to Claim 23, wherein the concentration of said antibody is 100 – 150 mg/ml.

(IX) EVIDENCE APPENDIX

Appendix of evidence submitted pursuant to §§ 1.130, 1.131, or 1.132 of this title or of any other evidence entered by the Examiner and relied upon by appellant in the appeal, along with a statement setting forth where in the record that evidence was entered in the record by the Examiner. Copies of the evidentiary documents are attached.

Reference/Exhibits	Entered in the Record
1. Sridhar et al., "Inhibitors of epidermal-growth-factor receptors: a review of clinical research with a focus on non-small-cell lung cancer." *The Lancet Oncology.* Volume 4, 397-406, 2003.	Cited by the Examiner in the Office Action mailed September 9, 2008. A copy of the reference was provided and entered on the record. Reliance thereon maintained in the Office Action mailed March 19, 2009.
2. Arvinte et al., "Stable Liquid Formulations of Antibodies." WO publication No. 02/096457.	Cited by the Examiner in the Office Action mailed September 9, 2008. A copy of the reference was provided and entered on the record. Reliance thereon maintained in the Office Actions mailed March 19, 2009.
3. Lam et al. "Antibody Formulation." US Patent No. 6,171,586.	Cited by the Appellant in the IDS filed July 11, 2008. A copy of the reference was provided and entered on the record. Acknowledged by the Examiner in the Office Action mailed September 9, 2008.

(X) RELATED PROCEEDINGS APPENDIX

(None)

Inhibitors of epidermal-growth-factor receptors: a review of clinical research with a focus on non-small-cell lung cancer

Srikala S Sridhar, Lesley Seymour, and Frances A Shepherd

Despite aggressive surgical and chemotherapeutic interventions, non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related death in men and women with overall cure rates of less than 15%. Recent advances in our understanding of cellular signalling and its critical role in tumorigenesis has led to the development of novel therapies which may offer new hope. In particular, the epidermal growth-factor receptor superfamily is an attractive therapeutic target because it is commonly overexpressed in malignant disease, regulates many vital cellular processes, and seems to be a negative prognostic indicator. Several selective inhibitors of this family of receptors are currently being evaluated in several cancers including NSCLC. In this review we examine current preclinical and clinical evidence on monoclonal antibodies (cetuximab, ABX-EGF, EMD72000, MAb ICR62, h-R3, MDX-447, MDX-H210, trastuzumab, and 2C4), immunoconjugates (Y10, Ua30:2, Mab806), anti-EGF vaccine (YMB2000), and tyrosine kinase inhibitors (gefitinib, erlotinib, CI1033, GW572016, EKB 569, PKI166, PD158780, and TAK 165).

Lung cancer is now the leading cause of cancer death in men and women in Europe and North America.[1] Although cytotoxic chemotherapy can relieve symptoms, improve survival, and occasionally contribute to cure in patients with localised disease, most individuals with lung cancer eventually relapse and die from either progressive local or metastatic disease.[2] During the past decade, our knowledge of the physiological role of growth factors and their receptors and their potential relevance to the pathogenesis of human cancer has greatly increased, leading to the development of novel targeted biological therapies.

One potential therapeutic target is the epidermal growth-factor receptor (EGFR) superfamily. These receptors are widely expressed cell-surface molecules implicated in the development and progression of cancer through effects on cell-cycle progression, apoptosis, angiogenesis, tumour-cell motility, and metastasis.[3–6] Overexpression of EGFRs correlates with a worse clinical outcome in several cancers including non-small-cell lung cancer (NSCLC), and tumours of the prostate, breast, stomach, colon, ovary, and head and neck, further supporting their role in tumorigenesis.[3–6] Many different strategies to interfere with EGFR-mediated signalling are being investigated and will hopefully translate into safe and effective treatments, especially in NSCLC where current therapies rarely offer a cure.
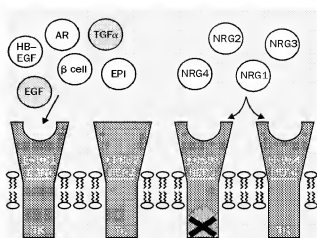

Figure 1. The EGFR superfamily of receptors.

EGFRs and their ligands

The EGFR family is made up of four distinct, but structurally similar, tyrosine kinase receptors encoded by the proto-oncogenes c-ERBB1/EGFR/EGFR1 (commonly referred to as EGFR), c-ERBB2/HER2 (commonly referred to as HER2), c-ERBB3/HER3, and c-ERBB4/HER4. In general, the receptors possess extracellular ligand binding domains, transmembrane domains, and intracellular tyrosine kinase domains (figure 1). HER3 has little or no tyrosine kinase activity compared with the other receptors,[5] and HER2 has strong tyrosine kinase activity, but no known cognate ligand. HER2 therefore serves as a co-receptor, forming heterodimers with other types of EGFRs resulting in augmented signal transduction on ligand binding.[5]

Several endogenous ligands for EGFRs are known, but the most important stimulatory ligands are epidermal growth factor (EGF) and transforming growth factor α (TGFα). After

SSS is a Clinical Research Fellow in Medical Oncology and FAS is a Professor of Medicine; both are at the Division of Medical Oncology, Department of Medicine of the University Health Network, Princess Margaret Hospital and the University of Toronto. LS is the co-ordinator of the Investigational New Drug Program of the National Cancer Institute of Canada Clinical Trials Group and Queen's University, Ontario, Canada.

Correspondence: Dr Frances A Shepherd, Princess Margaret Hospital, Suite 5104, 610 University Avenue, Toronto, ON, M5G 2M9. Tel: +1 416 946 4522. Fax: +1 416 416 6546. Email: frances.shepherd@uhn.on.ca
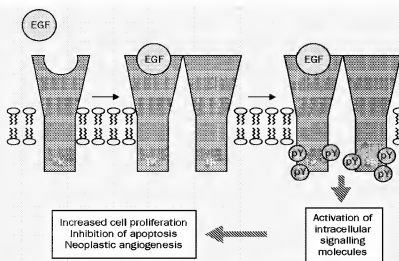
Figure 2. EGF binds to the receptor resulting in dimerisation and autophosphorylation. Dimerisation occurs between identical receptors (homodimers) or between two different members of the EGFR superfamily (heterodimers).

ligand binding, the receptor undergoes dimerisation, forming either homodimers or heterodimers, followed by internalisation of the receptor–ligand complex and tyrosine autophosphorylation.[5] These events ultimately trigger a cascade of physiological responses affecting cell proliferation and survival, angiogenesis, and potentially metastasis (figure 2).

Several aspects of the EGFR family signalling system are abnormal in malignant cells. For example, in NSCLC and breast cancer the *EGFR* and *HER2* genes are frequently amplified and the receptor gene products are overexpressed.[5,7] It is estimated that between 40% and 80% of NSCLCs overexpress EGFR, and 20–30% overexpress HER2.[6–11] This overexpression has been linked to poor overall outcome (figure 3).[12,13]

EGFR also exists in a mutant form, EGFRvIII, which is the result of a 267-aminoacid inframe deletion and insertion of a glycine in the fusion junction of the extracellular domain. This mutation is detected in about 15% of NSCLCs and in other solid tumours, and leads to ligand-independent constitutive tyrosine kinase activity, altered subcellular localisation of the receptor, and may confer resistance to chemotherapy.[14]

Autocrine overproduction of EGF and TGFα may also promote tumour formation and progression. Some tumours that overexpress EGFR may also overexpress TGFα, although there is no absolute association between their simultaneous overexpression.[5] This evidence suggests that some tumours have the potential for

multifactorial modulation of signalling through EGFR via control of both the receptor and its ligand.

Understanding the EGFR superfamily and their ligands has lead to the development of new therapeutic strategies including monoclonal antibodies, vaccines against EGF, ligand-toxin conjugates, and tyrosine kinase inhibitors. In this review we discuss the results of research to date with particular emphasis on studies in patients with NSCLC.

Monoclonal antibodies

Monoclonal antibodies have been developed that target different members of the EGFR superfamily. They are highly specific with few side-effects and may be synergistic with chemotherapy and radiation. The agents that fall into this category include antibodies to EGFR and monoclonal antibodies against HER2, truncated monoclonal antibody fragments (scFv), and fusion ligands conjugated with toxins and antisense oligonucleotides (tables 1 and 2).

Antibodies to EGFR

Cetuximab (IMC-C225) is a human–murine chimeric IgG monoclonal antibody that competitively binds to the extracellular domain of EGFR, preventing tyrosine kinase activation, inhibiting cell growth, and in some cases inducing apoptosis.[15] Preclinical studies show that cetuximab inhibits
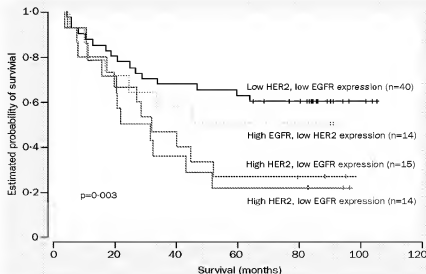

Figure 3. Estimated probability of survival of patients with non-small-cell lung cancer who underwent resection versus combined patterns of EGFR and HER2-neu co-expression. The median survival was 45·47 months in the low EGFR-expression group, 31·10 months (95% CI, 14·77–47·43) in the high HER2-neu-expression group, and 22·03 months (95% CI, 2·30; 41·76; p=0·003) in the high HER2-neu and EGFR-expression group.

the proliferation of cell lines expressing EGFR, and increases the cytotoxic activity of chemotherapy and radiation.[15,16] Cetuximab alone and in combination with chemotherapy or radiotherapy was generally well tolerated in phase I trials; fever, asthenia, nausea, elevation of liver enzymes, and allergic and acneiform skin reactions were reported to be the major toxic effects.[16,17] The acneiform rash that is a characteristic side-effect of many EGFR-targeted therapies does not preclude continued cetuximab treatment, and its presence may even predict the subgroup of patients who respond to treatment.[16,18] Cetuximab in combination with chemotherapy has shown activity in head and neck and colorectal cancers with acceptable toxic effects.[18–20] Phase II trials of cetuximab combined with gemcitabine and carboplatin, paclitaxel and carboplatin, and single-agent docetaxel in patients with NSCLC have all shown it is possible to combine cetuximab safely with chemotherapy.[21–23] In the two first-line trials, the 28·6% response rate in the gemcitabine study[21] and the 29% response rate in the paclitaxel study[22] do not appear to be higher than would be expected with chemotherapy alone. Similarly, in the second-line setting, cetuximab and docetaxel resulted in a 22·3% overall response rate, but the median survival was only 7·5 months.[23] A small randomised phase II trial compared chemotherapy with vinorelbine and cisplatin to the same chemotherapy with cetuximab in the first line treatment of NSCLC.[24] Overall response rates favoured the

Table 1. Monoclonal antibodies that target EGFRs

Monoclonal antibody	Properties	Trial status	Regimen	Tumour type
Cetuximab	Anti-EGFR	Phase II	Monotherapy	NSCLC, renal, head and
		Phase III	Combination	neck, colon
ABX-EGF	Anti-EGFR	Phase I	Monotherapy	NSCLC, renal, oesophagus,
		Phase II	Combination	pancreas, prostate
EMD72000	Anti-EGFR	Phase I	Monotherapy	Head and neck, oesophagus colon, cervix
MAb ICR62	Anti-EGFR	Phase I	Monotherapy	NSCLC, head and neck
h-R3	Anti-EGFR	Phase I	Combination	Head and neck
MDX-447	Bispecific Anti-EGFR	Phase I/II	Monotherapy	
MDX-H210	Bispecific Anti-HER2	Phase II	Monotherapy Combination	
Trastuzumab	Anti-HER2	Phase I	Combination	NSCLC, breast
		Phase III		
2C4	Anti-HER2	Phase I	Monotherapy	

cetuximab group (53·3% vs 32·2%), as did disease control rates (93·3% and 77·4%). Progression-free survival and overall survival have not been reported to date. A phase III trial testing use of cisplatin with or without cetuximab in patients with advanced head and neck cancer showed an increased response rate with the addition of cetuximab but this did not translate into an increase in progression-free survival (table 3).[25]

ABX-EGF is a fully humanised IgG2 monoclonal antibody with a higher binding affinity for EGFR than cetuximab. It inhibits tyrosine phosphorylation in a dose-dependent manner because it blocks the EGF binding site on the receptor and causes rapid internalisation of EGFR.[26] In xenograft

Table 2. Other compounds that target the EGFRs

Class of compound	Compound	Properties	Trial status	Regimen	Tumour type
Immunoconjugates	MAb 528 plus Rnase	Anti-EGFR	Preclinical		
	Cetuximab/ricin A	Anti-EGFR	Preclinical		
Anti-EGFRvIII	Y10	Anti-EGFRvIII	Preclinical		
	Ua30:2	Anti-EGFRvIII	Preclinical		
	MAb806	Anti-EGFRvIII	Preclinical		
Vaccine against EGF	YMB2000	Recombinant EGF plus recombinant p84 protein	Phase I Phase II	Monotherapy	NSCLC
Tyrosine kinase inhibitors	Gefitinib	Anti-EGFR	Phase II Phase III	Monotherapy Combination	NSCLC, gastric, prostate, breast, others
	Erlotinib	Anti-EGFR	Phase III	Monotherapy Combination	NSCLC, pancreas
	CI1033	All EGFR family receptors	Phase II	Monotherapy Combination	
	GW572016	Anti-EGFR Anti-HER2	Phase I	Monotherapy	
	EKB569	Anti-EGFR Anti-HER2	Phase I	Monotherapy	
	PKI166	Anti-EGFR Anti-HER2	Phase I Phase II	Monotherapy Monotherapy	
	PD158780	All EGFR family receptors	Preclinical		
	TAK165	Anti-HER2	Phase I	Monotherapy	

models, ABX-EGF resulted in complete eradication of some tumours with high EGFR expression. ABX-EGF may also be synergistic with chemotherapy.[27] In a phase I trial of 43 patients with NSCLC, dose-dependent acneiform skin rash was transient and biological activity was observed even at low doses.[28] Phase II trials with ABX-EGF in patients with kidney, colorectal, prostate, and lung cancer are underway. A trial combining ABX-EGF with paclitaxel and carboplatin is being done in patients with advanced NSCLC.

EMD 72000, a humanised monoclonal antibody that selectively binds EGFR, has shown antiproliferative effects against head and neck squamous carcinoma cell lines.[29] In murine models, the extent of tumour regression after treatment with EMD 55900 (the murine version of the antibody) correlated directly with the extent of EGFR expression. Also, in mice treated simultaneously with TNFα (0·5 μg/g) and EMD 55900 or 72000, increased antitumour effects including complete tumour eradication were observed.[30] In phase I trials in patients with tumours expressing EGFR, the maximum tolerated dose of EMD 72000 on a weekly schedule was 1600 mg per week. Headache and fever were dose limiting at higher doses. Of 158 patients evaluable for tumour response, five showed partial remission, and four had stable disease.[31]

MAb ICR62 is a rat monoclonal antibody that blocks binding of EGF and TGFα to EGFR. In vitro, it inhibits growth of tumour cells that overexpress EGFR and in xenograft models eradicates EGFR-expressing tumours.[79] MAb ICR62 has also been shown to have additive effects when given in combination with cisplatin.[79] In a phase I trial of 20 patients with squamous-cell cancers of lung and head and neck that expressed EGFR, no serious toxic effects were observed with doses up to 100 mg a day. Four patients showed human anti-rat antibody (HARA) responses, and biopsy samples taken from four patients who received doses of MAb ICR62 of 40 mg or greater showed localisation of the antibody to tumour-cell membranes.[32]

h-R3 is a humanised monoclonal antibody (IgG1), directed against EGFR. In xenograft models of human lung adenocarcinoma, radiolabelled h-R3 was preferentially taken up into tumour tissue more than normal tissue.[33] A phase I dose escalation study of h-R3 in patients with locally advanced head and neck cancer showed that the antibody was well tolerated and that it may act synergistically with radiation therapy.[34] With recent advances in radioimmunotherapy techniques, h-R3 may be useful in targeting radiotherapy specifically to tumour sites.

The class I IgG receptor or CD64 receptor on cytotoxic effector cells can initiate the destruction of tumour cells. MDX-447 is a bispecific antibody comprised of humanised Fab anti-CD64 and humanised Fab anti-EGFR.[35] In vitro, MDX-447 recognises the CD64 receptor and EGFR, thereby targeting cytotoxic effector cells to tumour cells

expressing EGFR, resulting in cell lysis.[36] MDX-447 given alone and with granulocyte colony-stimulating factor is being evaluated in phase I/II trials of patients with tumours that overexpress EGFR. Main toxic effects include fever, chills, blood pressure lability, and myalgia. Of 36 evaluable patients, nine had stable disease for 3–6 months. The optimum dose and the maximum tolerated dose have yet to be defined.[35] MDX-H210, is another bispecific antibody with humanised Fab anti-CD64 and humanised Fab anti-HER2.[35] Three phase II trials of MDX-H210 are being done in patients with tumours that overexpress HER2.

Antibodies to HER2

Trastuzumab, a monoclonal antibody against the extracellular domain of HER2, was originally developed for use in breast cancers, in which overexpression of HER2 occurs most frequently, generally because of gene amplification.[7] HER2 is a marker of more aggressive disease, lower rates of oestrogen-receptor expression, higher rates of recurrence, and a worse overall prognosis.[8,37] A phase III trial of first-line treatment of patients with metastatic breast cancer with overexpression of HER2 found that the addition of trastuzumab to chemotherapy was associated with a longer time to disease progression (4·6 months vs 7·4 months), higher objective response rate (32% vs 50%), lower death rate at 1 year (33% vs 22%), and longer median survival (20·3 months vs 25·1 months; table 3). Cardiac dysfunction was seen in patients receiving simultaneous trastuzumab and anthracyclines.[7,8]

On the basis of these encouraging results, trastuzumab was evaluated in patients with NSCLC overexpressing HER2—overexpression is less frequent and believed to be the result of polysomy of chromosome 17 and not the gene amplification seen in breast cancer.[38] HER2 overexpression in NSCLC is most commonly found in adenocarcinomas and large-cell carcinomas and is predictive of poorer outcomes (figure 2).[8–12] In a randomised trial of patients with HER2-positive advanced NSCLC, the addition of trastuzumab to gemcitabine and cisplatin failed to increase response rates or increase progression-free or overall survival (table 3).[39] However, only 2% of patients with NSCLC who were

Table 3. Results of trials of monoclonal antibodies directed against EGFRs

Tumour type	Regimen	Response rate (%)	Time to progression (months)	Progression-free survival (months)	Ref
Breast	Chemotherapy*	32	4·6	6·1	17
	Chemotherapy plus trastuzumab	50	7·4	9·1	
Head and neck	Cisplatin	9·3	··	3·4	25
	Cisplatin plus cetuximab	22·6	··	3·4	
Lung	Gemcitabine/cisplatin	41	7·2	7·0	39
	Gemcitabine/cisplatin plus trastuzumab	36	6·3	6·1	
Lung	Vinorelbine/cisplatin	32·2	··	··	24
	Vinorelbine/cisplatin plus cetuximab	53·3	··	··	

*Chemotherapy consisted of an anthracycline (doxorubicin 60 mg/m² BSA or epirubicin 75 mg/m²) plus cyclophosphamide (600 mg/m²) for patients who had never received an anthracycline or paclitaxel (175 mg/m²) for patients who had received postoperative anthracyclines.

screened for entry into this trial were found to have HER2-positive tumours by fluorescence in-situ hybridisation (FISH), which is superior to immuno-histochemistry in predicting response to trastuzumab in breast cancer.[37] Five of the six patients who had HER2-positive tumours (as determined with FISH) responded to treatment. Nonetheless, it seems that trastuzumab is unlikely to improve treatment outcome for most patients with NSCLC.

2C4 is an antibody against the ectodomain of HER2 at a site distinct from that of trastuzumab. It acts specifically by inhibiting the association of HER2 with other members of the EGFR superfamily and it does not cross react with trastuzumab. Preclinical information suggests that 2C4 will inhibit the growth of both androgen-dependent and androgen-independent prostate tumours grown as xenografts in athymic mice. A phase I study of 2C4 given every 3 weeks is being done.[40]

Conjugates of the monoclonal antibody to EGFR MAb 528 and mammalian pancreatic ribonuclease (an endogenous protein possessing antitumour activity) have been evaluated in preclinical studies. This immunoconjugate showed dose-dependent cytotoxicity against EGFR-expressing squamous cancer cells but not against EGFR-deficient small-cell-lung cancer cells.[41] Immunoconjugates of cetuximab and the ricin A chain (a potent inhibitor of protein synthesis) and EGF and Pseudomonas endotoxin are also at the preclinical stage.[42]

EGFRvIII has a constitutively active tyrosine kinase and unlike wild-type EGFR, does not bind ligand or undergo receptor dimerisation.[43,44] Since it is preferentially expressed in tumour tissue, EGFRvIII may serve as a highly specific target for therapy. A murine homologue of human EGFRvIII has been created and Y10—an IgG2a murine monoclonal antibody that recognises the human and murine equivalents of this variant receptor—has been investigated.[45] In that in-vitro study, Y10 was found to inhibit DNA synthesis and cellular proliferation, and induce complement-mediated and antibody-dependent cell-mediated cytotoxicity; phase I trials have not yet begun.[45] Ua30:2, another antibody to EGFRvIII has been studied in glioma tissue sections and has shown no measurable cross-reactivity with wild-type EGFR. However, clinical evaluation of this agent has not yet begun.[45] MAb806, also an antibody to EGFRvIII, is being evaluated in preclinical studies.[46] A phase I randomised study of an EGFRvIII peptide vaccine with granulocyte macrophage colony-stimulating factor vs keyhole limpet haemocyanin as adjuvant therapy in patients with EGFRvIII-expressing cancers is being done.

Vaccination against EGF
Another novel approach against the EGFR signal transduction cascade involves inducing an active immune response against the EGF ligand.[47] YMB2000 is an EGF vaccine that is a conjugate of recombinant EGF (rEGF) made in yeast, and recombinant P64K protein made in Escherichia coli. rEGF alone is not antigenic but in preclinical studies of rEGF

conjugated to P64K an immune response to both proteins has been observed.[48] Although there is a theoretical risk of inducing autoimmunity, human safety and immunogenicity studies have shown seroconversion rates of 60% without evidence of significant toxic effects. Secondary reactions were mild and limited to erythema and itching at the site of injection.[47] Patients who developed a high antibody response showed a trend towards improved survival. There is currently a randomised phase II study assessing the safety and immunogenicity of human EGF vaccine in patients with stage III/IV NSCLC. Secondary objectives include the preliminary assessment of efficacy (survival benefit, objective response) and quality of life.

Tyrosine kinase inhibitors
The most active tyrosine kinase inhibitors are small molecules that compete with and prevent binding of adenosine triphosphate to the intracellular tyrosine kinase region. These agents cause tumour regression by increasing apoptosis and by inhibiting cellular proliferation and angiogenesis. The two compounds that are at the most advanced stage of development are gefitinib and erlotinib both of which target EGFR.

Gefitinib
Gefitinib (ZD1839, Iressa) is an oral EGFR-specific anilinoquinazoline which reversibly inhibits autophos-phorylation, resulting in reduced c-FOS mRNA—a transcription factor forming part of the AP1 complex—and a shift of cells from S phase into G0/G1.[49] Preclinical studies showed that gefitinib can inhibit and even induce complete regression of well-established A431 xenografts and potentiate the cytotoxic effects of ionising radiation and chemo-therapy.[50–51] In phase I studies of gefitinib given in doses of 150–1000 mg per day, the most frequent adverse events were nausea, vomiting, an acneiform rash, and diarrhoea, the latter two effects becoming dose limiting at the maximum tolerated dose of 800 mg per day.[52] The skin is particularly susceptible to the effects of gefitinib largely because the EGFR is highly expressed in keratinocytes and in cells of eccrine and sebaceous glands.[54] Antitumour activity was observed at all dose concentrations but there was no evidence of a dose-response association. The doses chosen for phase II/III investigations were 250 mg and 500 mg per day, at which antitumour activity and pharmacokinetics consistent with preclinical activity were common.[55]

Table 4. Results of trials of second-line and third-line treatment of non-small-cell lung cancer

Regimen	Response rate (%)	Time to progression (months)	Median survival (months)	1-year survival (%)	Ref
Gefitinib 250 mg	18	2·7	7·6	..	51
Gefitinib 500 mg	19	2·8	8·1	..	(IDEAL 1)
Gefitinib 250 mg	12	..	6·1	29	52
Gefitinib 500 mg	9	..	6·0	24	(IDEAL 2)
Best supportive care	0	1·6	4·6	19	53
Docetaxel 75 mg/m²	6	2·7	7·0	29	
Erlotinib	14	2·1	9·0	40	58

Table 5. Results of trials of gefitinib in the first-line treatment of non-small-cell lung cancer

Dose	Response rate (%)	Median survival (months)	1-year survival (%)	Ref
Placebo	44·8	11·1	45	54*
Gefitinib 250 mg	50·1	9·9	42	(INTACT 1)
Gefitinib 500 mg	49·7	9·9	44	
Placebo	33·6	9·9	42	55†
Gefitinib 250 mg	35·0	9·8	42	(INTACT 2)
Gefitinib 500 mg	32·1	8·7	38	

*Compared with gemcitabine and cisplatin. †Compared with paclitaxel and carboplatin.

The results of two large randomised phase II trials, (IDEAL 1 and 2) have now been reported. In the IDEAL 1 trial, 210 patients with NSCLC who had failed one or two chemotherapy regimens (at least one platinum-based therapy) were randomly assigned to receive 250 mg or 500 mg per day of gefitinib. There were no differences between the two doses with respect to response rate, time to progression, or median survival. Response rates were also similar whether gefitinib was used as second-line (17·9%) or third-line treatment (19·8%). The frequency of rash and diarrhoea were greater in patients who received 500 mg per day.[55] In the IDEAL 2 study, 216 patients who had failed two or more chemotherapy regimens containing platinum and docetaxel received 250 mg or 500 mg gefitinib per day. The response rates were 12% and 9% and median survival was 6·1 and 6·0 months, respectively. An increased incidence of adverse events in patients receiving 500 mg per day was also seen.[56] The results are presented in table 4 and can be compared with results from the TAX 317 trial in which patients with stage IIIB/IV NSCLC who had failed cisplatin-based chemotherapy were randomly assigned to receive best supportive care or 75 mg/m² docetaxel.[57] Compared with the results that can be achieved with second-line chemotherapy, the response rates, survival, and toxicity profiles in the IDEAL 1 and 2 trials are very encouraging. On the basis of these two trials, gefitinib was approved in Japan in 2002, and more than 20 000 patients have now been treated. Interstitial lung disease has been reported from Japan as an unexpected side-effect of gefitinib and is currently being investigated further. However, an analysis of the incidence of interstitial lung disease in the patient databases of the two placebo-controlled first-line trials in NSCLC[58,59] has shown that the rates of this toxic effect were the same in the active treatment and the placebo groups (0·9% and 1·1%, personal communication, George Blackledge and Steve Averbuch, AstraZeneca). These observations suggest that interstitial lung disease may not be a toxic effect that is specifically related to gefitinib therapy.

Two randomised, placebo-controlled, phase III trials (INTACT 1 and 2) of gefitinib in chemotherapy-naive patients with stage IIIB/IV NSCLC have been reported (table 5). The trials combined gefitinib (250 or 500 mg per day) or placebo with either gemcitabine and cisplatin (INTACT 1) or paclitaxel and carboplatin (INTACT 2).[58,59] In both trials the toxic effects of gefitinib combined with chemotherapy were comparable to chemotherapy alone, with the exception of

additive dose-dependent diarrhoea and skin rash. However, the results of the INTACT trials were disappointing because the addition of gefitinib to chemotherapy failed to show improved response or survival. An explanation for this result may be that chemotherapy and gefitinib are targeting the same cell population and that the chemotherapy response masks that of gefitinib. Alternatively, chemotherapy may directly or indirectly affect EGFR function or expression thereby reducing the effects of gefitinib. Finally inhibition of EGFR may reduce cellular proliferation, thereby making chemotherapy less effective.[58]

The European Organization for Research and Treatment of Cancer plans to study docetaxel (75 mg/m²) with and without gefitinib (250 mg per day) in patients with NSCLC who have failed one cisplatin-based regimen. The study will begin when phase I testing of the docetaxel and gefitinib combination is complete. Gefitinib is also being studied in patients with earlier stage NSCLC. An intergroup trial led by the Southwest Oncology Group is evaluating gefitinib in patients with inoperable stage IIIA/B NSCLC. Patients with stable or responsive disease after concurrent chemotherapy and thoracic radiation followed by consolidation docetaxel are randomly assigned to receive gefitinib or placebo as maintenance therapy for up to 5 years.

In a large intergroup trial led by the National Cancer Institute of Canada Clinical Trials Group (NCI-CTG), patients with completely resected stage IB, II, and IIIA NSCLC are being randomly assigned postoperatively to receive 250 mg per day gefitinib or placebo for up to 2 years. This trial aims to establish a tumour bank that can hopefully be used to answer some fundamental questions about patient selection for anti-EGFR therapy. Currently it is not known whether EGFR expression or overexpression is necessary for response or clinical benefit from treatment. Although trials in advanced disease will attempt to answer these questions, the studies may lack statistical power because not all patients have tumour samples available for analysis. A smaller trial of identical design is currently on hold in Japan pending further examination of the potential for gefitinib to cause interstitial lung disease.

Phase I and II studies of gefitinib with other chemotherapy regimens and with thoracic irradiation are ongoing in several centres. Gefitinib is also being evaluated in patients with premalignant dysplastic lesions detected at bronchoscopy to determine whether these changes may be reversible. Studies are also under development to evaluate gefitinib in other tumour types including breast cancer and head and neck cancer.

Erlotinib

Erlotinib (Tarceva, CP-358774, OSI 774) is another anilinoquinazoline derivative and orally active EGFR inhibitor that can induce both cell-cycle arrest in G1 and apoptosis. It inhibits EGFR autophosphorylation with a

selectivity more than 1000-times greater than other tyrosine kinase inhibitors and reduces EGFR-associated phosphotyrosine by about 70% 24 h after a single 100 mg/kg dose.[60] Erlotinib also interferes with signalling via the variant receptor EGFRvIII.[61] In mouse xenograft models, concurrent erlotinib and cisplatin chemotherapy produced increased antitumour activity over that of cisplatin alone, with no increase in toxic effects.[62] Phase I studies showed that diarrhoea, rash, nausea, headache, emesis, and fatigue were the most frequent side-effects. At doses of 200 mg per day, diarrhoea was dose limiting but manageable with loperamide or a reduction in dose to 150 mg per day. The 150 mg per day dose was selected for subsequent studies because of its safety and tolerability profile and pharmacokinetic parameters.[63] By contrast, the 250 mg and 500 mg doses of gefitinib chosen for clinical trials were much lower than the maximum tolerated dose for gefitinib, which is 800 mg per day, raising questions about appropriate dosing of anti-EGFR therapies.

In a phase II trial of erlotinib in patients with NSCLC who had been treated previously and whose tumours showed more than 10% EGFR expression, the most common adverse effect was a maculopapular acneiform rash. The response rate was 14%, time to progression was 2·1 months, median survival was 9·0 months, and 1-year survival was 40%. Tumour response and survival did not correlate with the extent of EGFR expression (table 4).[63] Survival in this erlotinib trial was similar to the C225 trial in that it correlated most closely with the development of rash.

The preclinical synergy of erlotinib with platinum-based chemotherapy and non-overlapping toxic effects provided the rationale for combining erlotinib with chemotherapy. Phase I studies of erlotinib with gemcitabine and cisplatin and paclitaxel and carboplatin showed that erlotinib could be safely added to these combinations at a dose of 100 mg per day.[63,64] Two trials of 1000 patients given gemcitabine and cisplatin and paclitaxel and carboplatin with or without erlotinib have completed accrual but results have not yet been published.

A randomised phase III trial of single-agent vinorelbine or erlotinib is under development for patients with poor performance status. The Eastern Co-operative Oncology Group is planning to evaluate docetaxel with or without erlotinib in patients with NSCLC who have failed one cisplatin-based regimen. The NCIC-CTG evaluated erlotinib in patients with NSCLC who had a poor performance status, patients declining second-line treatments, and as third-line treatment. Because there is no proven role for chemotherapy in these clinical settings, patients in this trial were randomly assigned at a ratio of 2:1 to receive erlotinib 150 mg per day or placebo. This trial completed accrual in February 2003 but results have not yet been published.

CI1033 (PD183805)

CI1033, another 4-anilinoquinazoline derivative, is a highly specific irreversible tyrosine kinase inhibitor that is unique because it targets all four members of the EGFR superfamily and the constitutively active variant form EGFRvIII. CI1033 inhibits receptor signalling by selectively binding to a specific cysteine residue in the ATP pocket of the kinase domain.[65] It does not however, interfere with other tyrosine kinase receptors, such as platelet-derived growth factor, basic fibroblast growth factor, or the insulin receptor, even at high concentrations.[65] Data from three phase I trials showed that the most common side-effects are emesis, diarrhoea, and rash but generally CI1033 is well tolerated.[66–70] A phase II study in patients with advanced ovarian cancer and a randomised phase II trial in patients with NSCLC after first or second-line chemotherapy evaluating different doses and schedules of CI1033 are being done.

GW572016

GW572016 is a 6-thiazolyquinazoline reversible kinase inhibitor of EGFR and HER2 kinases. In human xenograft studies, GW572016 has shown dose-dependent kinase inhibition, and seems to selectively target tumour cells overexpressing EGFR or HER2.[69] GW572016 has been tested in two phase I clinical trials in healthy patients. The most common adverse events were gastrointestinal symptoms, rash, and headache.[70,71]

EKB569

EKB569 is a 3-cyanoquinoline selective irreversible kinase inhibitor. It binds covalently and equipotently inhibits growth of cells overexpressing EGFR and HER2 but has little effect on cells with low levels of these receptors.[72] In a phase I trial, the most common adverse events were mild and reversible diarrhoea, rash, nausea, stomatitis, vomiting, and anorexia. At doses of 125 mg, grade 3 diarrhoea was dose limiting. EKB569 may be particularly effective clinically because it showed sustained tyrosine kinase inhibition that persisted even after the drug had cleared from the plasma.[73]

PKI166

PKI166, a pyrrolopyrimidine derivative, is a tyrosine kinase inhibitor that inhibits EGFR and HER2. It has antitumour activity in vivo in several EGFR overexpressing xenograft models. In nude mice implanted with human pancreatic carcinoma cells, PKI166 treatment reduced tumour volume by 45%, gemcitabine by 59%, and the combination of the two reduced tumour volume by 85%. Furthermore, mice given the combination treatment showed decreased lymph node and liver metastases and improved survival. A concomitant decrease in vascular endothelial growth factor and interleukin 8 was also observed suggesting that PKI166 has an antiangiogeneic effect.[74] Phase I trials of PKI166 have found elevations in liver enzymes to be dose limiting. Other less severe toxic effects included vomiting, diarrhoea, fatigue, and skin rash. The recommended phase II dose will be either 600 or 750 mg given in a 2-week on and off cycle.[75]

PD158780

PD158780 is a 4-[ar(alk)ylamino] pyridopyrimidine derivative that reversibly inhibits EGFR superfamily tyrosine kinases. Unlike gefitinib and erlotinib that target only EGFR, PD158780 is a potent inhibitor of auto and trans-phosphorylation of all four members of the EGFR superfamily. This agent is not yet in clinical trials.[76]

TAK165

TAK165 is a new tyrosine kinase inhibitor that targets HER2 specifically. In HER2 positive BT474 human breast tumours in mice, tumour regression was observed after 14 days of oral TAK165. This effect seemed to be mediated by inhibition of HER2 tyrosine kinase signalling and by inactivation of HER2 downstream molecules such as AKT, which is known to be antiapoptotic.[6] A phase I study of oral TAK165 given once daily to patients with advanced tumours that express HER2 is being done.[77]

Conclusion

During the past decade, several molecules that contribute to proliferation, invasion, and metastasis of cancer cells have been identified. Members of the EGFR superfamily are overexpressed in many tumours and are associated with poor prognosis. Therefore, they have become an important target for novel anticancer therapies, especially in the treatment of lung cancer where new and less toxic approaches are desperately required.

The two main classes of compounds specifically targeting EGFR include monoclonal antibodies and tyrosine kinase inhibitors. Cetuximab, an anti-EGFR antibody, has been found to control disease in patients with head and neck cancer and colorectal cancer in combination with chemotherapy. However, clinical trial results are not yet complete in patients with NSCLC. By contrast, trastuzumab, an anti-HER2 antibody that has shown significant clinical benefit in patients with breast cancer, is unlikely to have a major role in the treatment of NSCLC. The EGFR tyrosine kinase inhibitors gefitinib and erlotinib have shown promising antitumour activity against cisplatin-resistant NSCLC in phase I and phase II trials. However, results of recent phase III trials of gefitinib in combination with chemotherapy were disappointing and emphasise that we do not yet know the best way to incorporate this class of agents into our current treatment regimens.

Many questions remain unanswered regarding patient selection for EGFR-targeted therapy. It is not clear whether it is necessary for tumours to express or to overexpress the receptor for treatment benefit and some studies suggest that response to treatment correlates better with the development of the characteristic acneiform rash than with the level of EGFR expression. Furthermore, the best method for determining this expression has yet to be determined. Our increasing knowledge of the molecular biology of NSCLC may help to identify other biomarkers that will help predict response or resistance to novel biological agents.

Another important issue is dosage, duration, and schedule of anti-EGFR therapies. In contrast with conventional therapies, toxic effects and antitumour activity of biological therapies may not be linked and specific biological assays and pharmacologically guided studies may be required to determine the most effective dose. Duration of therapy has not been addressed in any trial to date, which may be of considerable importance if the trial of gefitinib in patients with stage IIIA inoperable NSCLC or the adjuvant trials in patients with early stage resected NSCLC are positive. Furthermore, it is also not known whether EGFR inhibitors

should be given concurrently with chemotherapy or used as maintenance therapy after cessation of chemotherapy in responding patients. The negative INTACT 1 and 2 trial results suggest that it might be appropriate to assess the latter approach in clinical trials. Finally, the use of combined anti-EGFR therapy with agents that target more than one member of the EGFR superfamily, combinations of EGFR targeting agents, or combinations with other biological agents with different mechanisms of action, have yet to be addressed clinically.

Most clinical trials to date with anti-EGFR agents have shown activity in preclinical settings in vitro and in vivo. However, as has been the case in the past with chemotherapy agents, the preclinical models have not always been able to reliably predict human clinical response. This discrepancy may be because of redundancy in EGFR signalling systems contributung to resistance, similar targets shared by chemotherapy and anti-EGFR agents masking clinical responses, or changes in receptor concentrations induced by chemotherapy. Alternatively, study design and study endpoints for biological therapeutic strategies may need to be adjusted in ways that facilitate better prediction of response. The generation of pretreatment and post-treatment tumour banks may enable us to better understand these therapies at the molecular level and to tailor treatment accordingly.

Perhaps the greatest issue surrounding the use of EGFR inhibitors will be the one of cost. Gefitinib, the only anti-EGFR agent to be licensed for NSCLC, was approved in Japan in July 2002. The cost in Japan is US$60 per 250 mg pill, which equates to a total cost of more than US$20 000 a year. Lung cancer is the second most common malignant disease in men and women in the Western world and this treatment alone could place a severe burden on the health-care systems of all countries regardless of whether the systems are publicly or privately funded or both. There have been no costing or cost-effectiveness studies of anti-EGFR therapy and these are needed urgently.

It is expected that EGFR-directed therapies will be established as effective novel treatments for patients with lung cancer and other malignant diseases once we understand how best to use them. For example, it has been postulated that in the elderly or those with poor performance status, anti-EGFR

monotherapy may be equally efficacious and better tolerated than conventional treatments. Clearly, considerable research is still required but the wealth of knowledge gained from these early biological therapy trials cannot be understated and these studies offer hope for new and effective therapies in the future.

Conflicts of interest

LS and FAS have received honoraria from and have stocks in AstraZeneca. FAS is a consultant for OSI Pharmaceuticals.

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(54) Title: STABLE LIQUID FORMULATIONS

(57) Abstract: The present invention provides stable liquid formulations of antibodies suitable for parenteral administration. Also provided are aqueous solutions which have high concentrations of therapeutical antibodies which may be used to produce therapeutical liquid formulations. The present invention also relates to uses, such as medical uses, of the stable liquid formulations and processes for the production of the stable liquid formulations.

STABLE LIQUID FORMULATIONS

Field of the Invention

The present invention relates to aqueous solutions which have high concentrations of
therapeutical antibodies and to stable liquid formulations which are based on such aqueous
solutions of antibodies. The present invention also relates to uses, such as medical uses, of
the stable liquid formulations and processes for the production of the stable liquid
formulations.

Background of the Invention

Stable liquid formulations of antibodies are useful for parenteral administration, such as
intravenous (i.v.), intramuscular (i.m.) or subcutaneous (s.c.) administration. Such
formulations must fulfill two key requirements: 1) the required drug concentration must be
achieved, and, 2) the drug must be chemically and physically stable in order to have a
sufficient shelf-life.

For a protein to remain biologically active, a formulation must preserve intact the
conformational integrity and at the same time the protein's multiple functional groups must
be protected from degradation. Degradation pathways for proteins can involve chemical
instability or physical instability. For example, chemical instability can result from
deamidation, hydrolysis, oxidation, beta-elimination or disulfide exchange, while physical
instability can result from denaturation, aggregation, precipitation or adsorption, for example.
Aggregation is one of the most common protein degradation pathways.

Most current stable formulations of antibodies are not liquid formulations. For example,
WO97/04801 describes a stable lyophilized formulation of anti-IgE antibodies.
The stability of proteins in aqueous formulations is of general importance to the
pharmaceutical industry. The problem has been addressed by drying the protein, for
example, by the method of freeze-drying. For a patient who needs daily injections of an
antibody, it is of importance that the product is easy to handle, to dose and inject. Because a
dried antibody formulation is then distributed and stored in dried form, the patient or medical

professional has to reconstitute the dried powder in a solvent before use, which is an inconvenience for the patient.

Thus, it is advantageous to provide a liquid antibody formulation for which reconstitution before use is not required.

Furthermore, the freeze-drying process is a costly and time consuming process, and it would be advantageous if this step could be avoided when preparing a commercial antibody formulation.

It would also be advantage for the manufacture and formulation of a therapeutical product if the final pharmaceutical solution contained only few or no additives.

Thus, there is a demand on the market for stable, liquid, injectable antibody formulations; and, in particular, for highly concentrated stable, liquid, injectable antibody formulations.

There is also a need for stable aqueous solutions comprising a high concentration of antibody protein that can be used as a starting material or intermediate in process to obtain stable liquid antibody formulations of the invention.

Brief summary of the invention

The present invention provides a stable aqueous solution comprising an antibody at a concentration of at least 50 mg/ml, and further comprising at least one acidic component.

Further, there is provided a suitable delivery system which contains the aqueous solution.

Further provided are the uses of the aqueous solution in a nasal spray or a slow release formulation.

Also provided is the use of the aqueous solution in a drying or freeze-drying process.

Stable aqueous solution are provided which can be used as an intermediate for the formulation of therapeutical formulations, e.g. further pharmaceutically acceptable components can be added to the aqueous solution in order to obtain the final therapeutical

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formulation. However, the stable aqueous solution of the invention can itself be used as a therapeutical formulation; i.e. including no or only few further additives.

Further components which may be added to the stable aqueous solution of the invention can be mere pharmaceutical additives which are not therapeutically active, or they can be therapeutically active substances. Also, by-products may or may not be present in the aqueous solutions of the invention. Accordingly, the stable aqueous solutions of the invention may either comprise, consist essentially of, or consist of an antibody at a concentration of at least 50 mg/ml and at least one acidic component.

Processes of making a therapeutical formulation employing the aqueous solution of the invention are also provided.

Thus, in one aspect of the invention a process is provided for the preparation of a therapeutical liquid formulation comprising an antibody, wherein in a first step an aqueous solution including an antibody at a concentration of at least 50 mg/ml and at least one acidic component is prepared; and, in a second step, at least one pharmaceutically acceptable additive is added to said aqueous solution.

Furthermore, a process is provided for the preparation of a therapeutical liquid formulation comprising an antibody at a concentration of more than 50 mg/ml, wherein in a first step an antibody solution in a suitable buffer is concentrated to between about 10 mg/ml and about 50 mg/ml; in a second step, the concentrated solution obtained in the first step is diafiltered with an aqueous solution of at least one acidic component, optionally containing $MgCl_2$ and/or $CaCl_2$ and/or further suitable additives; and, in a third step, the solution obtained in the second step is further concentrated to a concentration of more than 50 mg/ml.

Also provided is a process for the preparation of a therapeutical liquid formulation comprising an antibody at a concentration of more than 50 mg/ml, wherein
- in a first step an antibody solution in a suitable buffer is concentrated to a concentration of between about 10 mg/ml and about 50 mg/ml;
- in a second step, the concentrated solution obtained in the first step is diafiltered with an aqueous solution of at least one acidic component;
- in a third step, the solution obtained in the second step is further concentrated to an

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intermediate concentration of between about 100 and 200 mg/ml, preferably between about 100 and 150 mg/ml;
- in a fourth step, the intermediate concentrated solution obtained in the third step is diafiltered with an aqueous solution of at least one acidic component and further containing $MgCl_2$ and/or $CaCl_2$ and/or further suitable additives,
 - in a fifth step, the solution obtained in the fourth step is further concentrated to a concentration of more than 150 mg/ml.

Detailed description of the invention

I. High concentration aqueous solution of antibody and liquid formulations

The present invention provides highly concentrated aqueous solutions of antibody and liquid formulations based thereon. The concentrated aqueous solutions of the invention include a therapeutical antibody and at least one acidic component. The aqueous solutions therefore generally have a pH below pH 7.0. They may or may not include further salts or additives. They may be used as an intermediate in a process to obtain a therapeutical liquid formulation of the invention, but they also may be suitable therapeutical liquid formulations themselves, i.e. without the addition of further pharmaceutically acceptable additives.

In one aspect the invention provides a stable aqueous solution comprising an antibody at a concentration of at least 50 mg/ml, and further comprising at least one acidic component. Preferred are concentrations of the antibody of at least 80 mg/ml, 100 mg/ml, 140 mg/ml, 160 mg/ml, 180 mg/ml, 200 mg/ml, 220 mg/ml, 250 mg/ml or even 300 mg/ml.

In developing a high concentration stable aqueous solution of antibody, the high viscosity of protein solutions has been identified as a major obstacle. For example, in physiological saline conditions or buffers at concentrations above 50 mg/ml antibody solutions, such as for example solutions of monoclonal antibody E25, can start to become viscous and/or turbid. The viscosity increases with protein concentration. The high viscosity of antibody solutions is a disadvantage from a medical point of view as, for example, reconstitution times may be as long as 30min for an antibody lyophilizate. Further, after reconstitution and injection of a dry formulation about 30% of an antibody may be left in the vial and in the syringe, which severely increases the treatment cost.

The present invention now provides means to obtain a stable liquid pharmaceutical formulation comprising antibodies, such as anti-IgE antibodies, with a high protein concentration and a low viscosity.

Although we do not wish to be limited by any theoretical speculation, one phenomenon that may contribute to the observed viscosity of aqueous antibody solutions is the self-association of the antibody, or "aggregation". Antibody aggregates can be soluble or insoluble and both forms of aggregates can be covalent or non-covalent. The aggregates can give opalescent solutions, but there can also be non-visible aggregation which only can be shown chemically.

In addition to increasing viscosity, aggregation can be detrimental in several ways. For example, covalent aggregation in protein formulations may be essentially irreversible and could result in the production of inactive species, which in addition also may be immunogenic. Non-covalent aggregation can lead to loss of activity due to precipitation.

A "stable" aqueous solution or liquid formulation within the meaning of the invention is one in which the antibody therein essentially retains its physical and chemical stability and integrity upon storage. Various analytical techniques for measuring protein stability are available in the art. Stability can be measured at a selected temperature for a selected time period. For rapid screening, a formulation may be kept at 40°C for 2 weeks to 1 month, at which time stability is measured. Where the formulation is to be stored at 2-8°C, generally the formulation should be stable at 30°C or 40°C for at least 1 month and/or stable at 2-8°C for at least 1 year. For example, in one preferred embodiment the aqueous solution of the invention has a stability of at least 1 year at about 4°C. The extent of viscosity and/or aggregation can be used as an indicator of protein stability. For example, a "stable" formulation may be one wherein less than about 10% and, preferably, less than about 5%, preferably less than about 2%, or even less than about 1% of the protein is present as an aggregate in the formulation. Aggregation can, for example, be measured by size exclusion chromatography.

The solutions of the invention are stable not only with regard to aggregation but also with regard to the chemical stability of the antibody. Chemical stability may, for example, be

measured by hydrophobic interaction chromatography (HIC), for example by HIC-HPLC after papain digestion. For example, after storage of at least 1 year at about 4°C the peak representing unmodified antibody in HIC-HPLC after papain digestion decreases no more than 20%, preferably no more than 10%, more preferably no more than 5% or even no more than 1%, as compared to the antibody solution prior to storage.

As the person skilled in the art will readily appreciate, there are other methods suitable to measure the stability of the solutions of the invention. For example, chemical stability may also be measured by capillary electrophoresis.

Chemical instability can impair the activity of the antibody in question. Examples of chemical instability are degradation of the antibody or changes in tertiary and/or quaternary structure of antibody molecules. In preferred embodiments the solutions and formulations of the invention lose less than 50%, preferably less than 30%, preferably less than 20%, more preferably less than 10% or even less than 5% or 1% of the antibody activity within 1 year storage under suitable conditions at about 4°C. The activity of an antibody can be determined by a suitable antigen-binding assay for the respective antibody.

The ability of an acidic component to produce a stable liquid antibody solution at high protein concentration can be determined by making up a solution including the acidic component to be tested and storing it for 24 hours at 22°C. For example, if after this time the solution remains clear the acidic component has stabilized the antibody and is one suitable for the use in an aqueous solution according to the present invention.

The degree of stability achieved depends on the acid used and on its concentration, the antibody concentration, and on the storage temperature. In general, the higher the concentration of the antibody and the higher the storage temperature, the shorter the time before aggregation occurs. In general higher antibody concentrations require higher concentrations of the acidic component.

Accordingly, it is found in the present invention that stable aqueous solutions and liquid formulations including antibodies having an acceptable viscosity for therapeutical applications can be made in the presence of specific acidic components.

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Preferably, the viscosity of the aqueous solution or liquid formulation of the invention is below 200 mPa·s, preferably below 100 mPa·s, preferably below 70 mPa·s, more preferably below 50 mPa·s, more preferably below 20 mPa·s or even below 10 mPa·s at a shear rate of γ = 100 (1/s). Another suitable shear rate to measure viscosity of antibody solutions is γ = 220 (1/s).

Such reduced viscosity allows for a aqueous solution or liquid formulation of the invention having a higher concentration of the respective antibody. Thus, advantageously, the same amount of antibody may be administered in a smaller volume. Also, such smaller volume, advantageously, may allow to produce pre-filled delivery devices that include the entire therapeutical dosage of the respective antibody. Also, if small volumes can be used, a liquid formulation need not necessarily be isotonic to avoid pain to the patient. However, in one preferred embodiment the aqueous solution of the invention is isotonic. By "isotonic" it is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

According to the present invention the acidic component and the amount of acid being used is so chosen as to achieve the desired viscosity and stability of the high concentrated protein solution. Suitable acids that may be chosen include organic and inorganic acids. Organic acids of the invention may be carboxylic acids, such as monocarboxylic, dicarboxylic, tricarboxylic, tetracarboxylic, hydrocarboxylic acids or phenols. Weak organic acids are preferred acids of the present invention, for example monocarboxylic organic acids having a pK-value between 3.0 and 6.0, preferably between 4.5 and 5.0. Preferred examples of acidic components of the invention are acetic acid, citric acid, oxalic acid, succinic acid, tartaric acid, lactic acid, malic acid, glycolic acid and fumaric acid. In a particularly preferred embodiment the acidic component included in the aqueous solution is acetic acid.

Preferably, the pH of said aqueous solution or liquid formulation is above pH 3, for example between pH 3 and pH 7, more preferably it is between pH 3 and pH 6, more preferably between pH 4 and pH6, or even between pH 5 and pH 6. In one preferred embodiment the pH is about pH 5.0 or about pH 6.0. Certain pH ranges are particularly preferred, for example, preferred is a pH below pH 6.0, or below pH 5.8, or below pH 5.6 or below pH 5.4,

and a pH that is above pH 4.0, or above pH 4.2, or above pH 4.4, or above pH 4.6 or above pH 4.8, or above pH 5.0.

Preferably the acidic component of the invention, such as acetic acid, is present in a final concentration of at least 0.001%, preferably at least 0.01%, more preferably between 0.01% – 0.2%. In one embodiment of the invention no additional buffering agent is present in the aqueous solution or liquid formulation of the invention. In another embodiment of the invention no sodium salt, such as for example sodium acetate is present in the aqueous solution or liquid formulation of the invention.

The concentration of the antibody, such as an anti-IgE antibody such as for example E25 (as defined hereinbelow), is above 50mg/ml, for example it may be between 100 and 200 mg/ml and can go up to 300 mg/ml. Preferred is a concentration of at least 80, 100, 140, 160, 180, 200, 220, 250 or even 300 mg/ml. One preferred range is between 100 and 220 mg/ml for injectable solutions. If a protein shall be delivered via the nasal or even the oral route, preferred concentrations are at least 250 mg/ml or even 300 mg/ml, as high concentrations are particularly desirable for the delivery via the nasal or oral route.

The aqueous solution or liquid formulation of the invention may also contain more than one antibody as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect the other antibody. The aqueous solution or liquid formulation herein may also include an additional therapeutical protein which is not an antibody. Such antibodies or proteins are suitably present in combination in amounts that are effective for the purpose intended. When including a further protein component in the aqueous solution, the total protein concentration should be taken into account when choosing the concentration of the acidic component.

In one aspect the present invention also provides for a stable aqueous solution consisting merely of an antibody at a concentration of at least 50 mg/ml and an acidic component. In another aspect the stable aqueous solution however may also consist essentially of an antibody at a concentration of at least 50 mg/ml and an acidic component, in particular it may further include by-product or therapeutically inactive additives.

Preferably, the aqueous solution or liquid formulation of the invention further includes $CaCl_2$ and/or $MgCl_2$. In a preferred embodiment the concentration of $CaCl_2$ is within the range of 50 -200mM, more preferably within 50-130mM, preferably 100-130mM, most preferably about 100mM. In another preferred embodiment the concentration of $MgCl_2$ is within the range of 50 -200mM, more preferably within 50-130mM, preferably 100-130mM, most preferably about 100mM. Stable aqueous solutions or liquid formulations including $MgCl_2$ are a particularly preferred embodiment of the present invention. In a further preferred embodiments these aqueous solutions or liquid formulations further include a detergent and/or a sugar.

II. Antibodies

The term "antibody" is used in a broad sense. The term "antibody" specifically covers monoclonal antibodies (including full length antibodies which have an immunoglobulin Fc region), antibody compositions with polyepitopic specificity, bispecific antibodies, diabodies, and single- chain molecules, as well as antibody fragments and/or derivatives such as, for example, Fab, F(ab')₂, and Fv fragments or other antigen-binding fragments. For example, an antibody derivative may be a PEGylated form of an antibody or antibody fragment.

In a preferred embodiment the antibody used in the aqueous solution of the invention has an isoelectric point between pH 6 and pH 8.

The term "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method or may be made by recombinant DNA methods. The "monoclonal antibodies" may also be isolated from phage antibody libraries.

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to

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another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

"Humanized" forms of non-human antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof, such as Fv, Fab, Fab', F(ab')$_2$ or other antigen-binding subsequences of antibodies, which contain minimal sequence derived from non-human immunoglobulin. Usually, humanized antibodies are human immunoglobulins in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species. In some instances, Fv framework region residues of the human immunoglobulin are replaced by corresponding non-human residues. Also, complementarity determining region (CDR) residues originating from the non-human species may be replaced by corresponding human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences.

In a particularly preferred embodiment the antibody or antibody derivative is selected from anti-IgE antibodies, such as E25, E26, E27 (described in WO99/01556 as rhuMAbE-25, rhuMAbE-26, and rhuMAbE-27, respectively) or their fragments and derivatives. Preferably the anti-IgE antibody is a humanized murine antibody or a fully human antibody. Most preferably the anti-IgE antibody is Omalizumab, which is also named "E25". Another preferred anti-IgE antibody is named "E26" as further defined hereinbelow.

Generally, anti-IgE antibodies are described in the prior art, and in greater detail in the International applications WO 93/04173 and WO 99/01556. For example, WO 99/01556 specifically describes Omalizumab, also named E25, in Figure 12, and in the sequences ID-No. 13-14. Antibody molecules comprising a E26 sequence are described in WO 99/01556 and are selected from the group of F(ab) fragment (Sequence ID Nos. 19-20), sFv fragment (Sequence ID No. 22) and F(ab)'$_2$ fragment (Sequence Nos. 24-25), in accordance to Figures 12-15. Within this invention, the terms E25 and E26 shall be construed accordingly. Preferably, the IgE antibodies of the instant invention do not result in histamine release from mast cells or basophils.

Furthermore, U.S. Patent 5,449,760 generally describes anti-IgE antibodies that bind soluble IgE but not IgE on the surface of B cells or basophils. Antibodies such as these bind to

soluble IgE and inhibit IgE activity by, for example, blocking the IgE receptor binding site, by blocking the antigen binding site and/or by simply removing the IgE from circulation. Additional anti- IgE antibodies and IgE-binding fragments derived from the anti-IgE antibodies are described in U.S. Patent 5,656,273. U.S. Patent 5,543,144 describes further anti- IgE antibodies that are suitable for this invention, in particular anti- IgE antibodies that bind soluble IgE and membrane-bound IgE on IgE-expressing B cells but not to IgE bound to basophils.

III. Aqueous antibody solutions including suitable additives (liquid formulations)

It has been surprisingly found that after the preparation of the highly-concentrated aqueous antibody acid solution according to the invention different ingredients can be added without a substantial increase in viscosity. The antibody acid solution can for example be mixed with sugars, detergents and/or other additives. Accordingly the present invention also describes methods suitable for the preparation of long-term stable liquid formulations of antibodies including such additives. Also provided are the aqueous solutions including such additives themselves.

A person skilled in the art will appreciate that a wide variety of excipients may be used as additives. Components that may be used as additives are e.g.:
  a) liquid solvents, co-solvents, e.g. an alcohol, e.g. isopropanol,
  b) sugars or a sugar alcohols, e.g. mannitol, trehalose, sucrose, sorbitol, fructose, maltose, lactose or dextrans,
  c) detergents, e.g. Tween 20, 60 or 80 (polysorbate 20, 60 0r 80)
  d) buffering agents, e.g. acetate buffer
  e) preservatives, e.g. benzalkonium chloride, benzethonium chloride, tertiary ammonium salts and chlorhexidine diacetate.
  f) isotoning agents, e.g. sodium chloride
  g) carriers, e.g. polyethylene glycol (PEG), recombinant human serum albumin
  h) antioxidants e.g. ascorbic acid and methionine
  i) chelating agents e.g. EDTA
  j) biodegradable polymers e.g. polyesters
  k) salt-forming counterions e.g. sodium

A "preservative" within the meaning of the invention is a compound which can be added to the diluent to essentially reduce bacterial action in the reconstituted formulation, thus facilitating the production of a multi-use reconstituted formulation, for example. For example, preservatives may advantageously be included in solutions suitable for nasal administration or in solutions for use with multiple pen injectors.

Preferred compounds to be added as further additives are detergents such as Tween 20, sugars such as sucrose, fructose, mannitol and preservatives. Preferably, additives derived from animal origin such as gelatine or serum albumin (e.g. BSA) are excluded from formulations of the invention.

Generally, acceptable additives are nontoxic to recipients at the dosages and concentrations employed. The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, alternatively, sterility of the entire mixture may be accomplished by autoclaving the ingredients, except for protein, at about 120°C for about 30 minutes, for example.

The percentage of the acid solution and the amount of additives used can vary and depends on the intended use. For example during different manufacturing steps the concentration of the acid solution can differ from the concentration of the final product.

It should be noted that certain additives such as ethanol, phosphate buffer saline (PBS), or citrate buffer, may induce gelation, increased viscosity and/or aggregation of the antibody in question under certain pH conditions. If the problems cannot be avoided by routine changes in pH, such additives should preferably not be used for preparing compositions of this invention.

A liquid formulation may, for example, be made by adding the additives to an aqueous solution of the antibody and then stirring to dissolve. Any suitable stirrer may be used, e.g. a vortex mixer. It is preferred to dissolve the antibody in an aqueous solution of the acid and then to add an aqueous solution of the additives. The stirring may preferably be carried out under an inert gas atmosphere, such as nitrogen or argon, and the resulting solution may preferably be degassed under vacuum. The inert gas atmosphere and degassing both may

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help to prolong the stability of the solution. After preparation the solution may be stored in glass or plastics containers.

Preferably, the aqueous solution or liquid formulation of the invention further includes $CaCl_2$ and/or $MgCl_2$. In a preferred embodiment the concentration of $CaCl_2$ is within the range of 50 -200mM, more preferably 50-130mM, preferably 100-130mM, most preferably about 100mM. In another preferred embodiment the concentration of $MgCl_2$ is within the range of 50 - 200mM, more preferably 50-130mM, preferably 100-130mM, most preferably about 100mM.

In one preferred embodiment the aqueous solution or liquid formulation of the invention further includes a detergent, such as for example Tween 20, Tween 60 or Tween 80.

In another preferred embodiment the aqueous solution or liquid formulation of the invention further includes at least one sugar. In a further preferred embodiment the aqueous solution or liquid formulation of the invention further includes at least one sugar selected from the group comprising trehalose, sucrose, mannitol, sorbitol, fructose, maltose, lactose or a dextran. However, in one embodiment of the invention the aqueous solution or liquid formulation of the invention does not include maltose.

In another embodiment the aqueous solution or liquid formulation of the invention further includes at least one buffering agent.

One desirable anti-IgE antibody aqueous solution discovered herein includes an anti-IgE antibody in amount between 100 and 200 mg/ml, preferably of about 190 mg/ml or of about 220 mg/ml, and $CaCl_2$ or $MgCl_2$ in an amount between 50 and 200mM, preferably of about 50 mM or of about 100 mM, optionally a buffer and optionally a detergent, such as a Tween 20, e.g. at a concentration of about 0.02%. Preferably, this anti- IgE formulation is stable at 8°C for at least 1 year.

IV. Devices

The aqueous solution or liquid formulation of the invention may, for example, be used with standard ampoules, vials, pre-filled syringes or multiple administration systems. In preferred embodiments, the aqueous solution may be administered to the patient by subcutaneous

administration. For example, for such purposes, the formulation may be injected using a syringe. However, other injection devices for administration of the formulation are available such as injector pens, and subcutaneous patch delivery systems such as, for example, chip devices. However, the aqueous solution may also be administered to the patient by inhalation devices. Conventional systems for delivery of molecules through the nasal passages and the lung include metered dose inhalers, and liquid jet and ultrasonic nebulizers.

Accordingly, in one aspect the present invention also provides a delivery system which contains the aqueous solution selected from the group of single use injection syringes or inhalation devices.

The delivery system comprises a container. Suitable containers include, for example, bottles, vials (e.g. dual chamber vials), syringes (such as dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the aqueous solution and the label on, or associated with, the container may indicate directions for use. The label may for example indicate that the aqueous solution is useful or intended for subcutaneous administration. The container holding the formulation may be a multi-use vial, which allows for repeat administrations (e.g. from 2-6 administrations) of the aqueous solution.

Accordingly, also provided is the use of the aqueous solution or liquid formulation according to the invention for the production of a delivery system for the use treatment of a disease.

In another embodiment of the invention, an article of manufacture is provided which contains the aqueous solution of the present invention and provides instructions for its use. Thus, an article of manufacture is provided herein which comprises:
a) container which holds a concentrated aqueous solution of an antibody; and
b) instructions for diluting the concentrated aqueous solution with a diluent to a protein concentration in the diluted formulation of at least about 50 mg/mL. The article of manufacture may further comprise a second container which holds a diluent (eg. bacteriostatic water for injection comprising an aromatic alcohol).

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The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

V. Specific formulations

In another aspect of the invention there is provided a slow release formulation comprising the aqueous solution or liquid formulation of the invention. Preferred is a slow release formulation selected from the group of polymeric nano or microparticles, or from gels.

In a particularly preferred embodiment the slow release formulation is a gel such as a hyaluronic acid gel.

Besides convenience, slow release formulations offer other advantages for delivery of protein drugs including protecting the protein over an extended period from degradation or elimination, and the ability to deliver the protein locally to a particular site or body compartment thereby lowering overall systemic exposure.

The present invention, for example, also contemplates injectable depot formulations in which the protein is embedded in a biodegradable polymeric matrix. Polymers that may be used are the homo- and co-polymers of lactic and glycolic acid (PLGA). PLGA degrades by hydrolysis to ultimately give the acid monomers and is chemically unreactive under the conditions used to prepare, for example, microspheres and thus does not modify the protein. After subcutaneous or intramuscular injection, the protein is released by a combination of diffusion and polymer degradation. By using polymers of different composition and molecular weight, the hydrolysis rate can be varied thereby allowing release to last from days to months.

In a further aspect the present invention provides a nasal spray comprising the aqueous solution or liquid formulation of the present invention.

VI. Uses and processes for preparation

In a further aspect of the invention the use of an acidic component for the preparation of an aqueous solution comprising an antibody having a concentration of at least 50 mg/ml is provided.

Also provided is a process for the preparation of a aqueous solution according to the invention, which process comprises admixing an antibody with an acidic component.

Also provided is a process for the preparation of a therapeutical liquid formulation comprising an antibody, wherein in a first step an aqueous solution including an antibody at a concentration of at least 50 mg/ml and at least one acidic component is prepared, and, in a second step, at least one pharmaceutically acceptable additive is added to said aqueous solution.

Also provided is a process for the preparation of a therapeutical formulation including an antibody, which process comprises adding an acidic component on the last purification step of the preparation of said antibody. Such last step may, for example, be an elution step, a buffer exchange step or a step comprising continuous diafiltration.

Furthermore, a process is provided for the preparation of a therapeutical liquid formulation comprising an antibody at a concentration of more than 50 mg/ml, wherein in a first step an antibody solution in a suitable buffer is concentrated to a concentration between about 10 mg/ml and about 50 mg/ml; in a second step, the concentrated solution obtained in the first step is diafiltered with an aqueous solution of at least one acidic component, optionally containing $MgCl_2$ and/or $CaCl_2$ and/or further suitable additives; and, in a third step, the solution obtained in the second step is further concentrated to a concentration of more than 50 mg/ml.

For example, the aqueous solution of at least one acidic component may be a solution of acetic acid, such as a solution of between about 0.01% and about 0.1% acetic acid. $MgCl_2$ and/or $CaCl_2$ may be present at a concentration within the range of 50 -200mM, preferably 50-130mM, more preferably 100-130mM, most preferably about 100mM. In a further preferred embodiments these aqueous solutions further include a detergent and/or a sugar.

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Also provided is a process for the preparation of a therapeutical liquid formulation comprising an antibody at a concentration of more than 50 mg/ml, wherein
- in a first step an antibody solution in a suitable buffer is concentrated to a concentration of between about 10 mg/ml and about 50 mg/ml;
- in a second step, the concentrated solution obtained in the first step is diafiltered with an aqueous solution of at least one acidic component;
- in a third step, the solution obtained in the second step is further concentrated to an intermediate concentration of between about 100 and 200 mg/ml, preferably between about 100 and 150 mg/ml;
- in a fourth step, the intermediate concentrated solution obtained in the third step is diafiltered with an aqueous solution of at least one acidic component containing $MgCl_2$ and/or $CaCl_2$ and/or further suitable additives; and,
- in a fifth step, the solution obtained in the fourth step is further concentrated to a concentration of more than 150 mg/ml.

The diafiltration is generally carried out at constant retentate volume, with at least 5 volumes, or preferably 8 volumes, of diafiltration buffer.

In a preferred embodiment a solution of $MgCl_2$ and/or $CaCl_2$ and/or further suitable additives may directly be added to the intermediate concentrated solution obtained in the third step of the above 5-step process. If $MgCl_2$ and/or $CaCl_2$ and/or further suitable additives are directly added, the fourth step (i.e. diafiltration with an aqueous solution of at least one acidic component containing $MgCl_2$ and/or $CaCl_2$ and/or further suitable additives) thereafter may be omitted if no further adjustment of the respective concentrations of the salts and/or additives is required. Generally, the 5-step process of the invention which adds the salts and/or additives only to an intermediate concentrated solution of antibody avoids the appearance of aggregates and/or turbidity in solutions of the process.

In one preferred embodiment, in the fourth step a concentrated aqueous solution of $MgCl_2$ (or $CaCl_2$), for example at concentration 1 M, is added directly into an ultrafiltration system, to give approximately the desired resulting concentration (for example 50 mM or 100 mM).

In preferred embodiments of the processes of the invention carboxylic acids, such as acetic acid, are employed as the acidic component. In preferred embodiments of these processes

no salt of a carboxylic is added in the process. In particular, in these embodiments it is preferred if no salt of the corresponding carboxylic acid is added.

VII. Medical uses

In one aspect, the present invention also provides the aqueous solution of the invention for use in medicine. In particular, the use of the aqueous solution for the manufacture of a medicament for the treatment of disease, such as for example an allergic disease, is provided.

The appropriate dosage of the protein will depend, for example, on the condition to be treated, the severity and course of the condition, whether the protein is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the protein, the type of antibody used, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards. The antibody may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question.

The uses for a formulation including an anti-IgE antibody, for example, include the treatment or prophylaxis of IgE-mediated allergic diseases, parasitic infections, interstitial cystitis and asthma, in particular allergic athma, allergic rhinitis and atopic dermatitis, for example. Depending on the disease or disorder to be treated, a therapeutically effective amount of the anti-IgE antibody may be administered to the patient.

In another aspect there is provided the use of the aqueous solution of the invention in a drying or freeze-drying process.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention.

Examples

Example 1:

Solutions of 40 mg/ml E25 in the production buffer (10 mM histidine buffer 10 % sucrose) were dialyzed against large volumes of water and of 0.01% acetic acid. The obtained E25 solutions, in water and in 0.01% acetic acid, were concentrated by filtration. The obtained E25 solution in water (99 mg/ml E25, pH 7.04) was much more viscous than the 0.01% acetic acid E25 solution (98 mg/ml E25, pH 5.4).

The beneficial effect of acetic acid in obtaining solutions with reduced viscosity was further documented. For example, 160 mg/ml E25 could be easily obtained in 0.1% acetic acid (final protein solution had a pH of 4.8) or solution of 183 mg/ml E25 in 0.01% acetic acid. A water solution of E25 of 170 mg/ml could also be prepared, but it was much more viscous than all the acetic acid solutions.

No chemical degradation was detected by capillary zone electrophoresis (CZE) after storing the solutions at 8°C for 10 days.

Example 2:

The buffer of a solution of 40 mg/ml E25 in the production buffer (10 mM histidine buffer 10 % sucrose) was exchanged in a diafiltration equipment to 0.1% acetic acid. After that the E25 solution was concentrated by ultrafiltration to 161 mg/ml. The solution was fluid, no aggregation or opalescence was observed. The recovery was very good, about 95%. This solution of 161 mg/ml was further concentrated by filtration through centrifugation using Centricone tubes. Fluid, clear solutions of E25 in 0.1% acetic acid with concentrations of 214 mg/ml and also 297mg/ml were obtained. The solutions can be easily handled through syringe needles and permit the development of a single use prefilled syringe with small volume (e.g., 0.5 ml to 1ml).

Example 3:

A solution of 40 mg/ml E25 in the final production buffer (containing 0.02% Tween 20) was dialyzed against 0.1% acetic acid. The resulted' E25 solution in 0.1% acetic acid (still containing Tween 20 detergent) was concentrated by filtration through centrifugation using Centricone: a concentration of 243 mg/ml E25 was reached. The solution fluidity was similar to the fluidity of the solutions without Tween 20, showing that the detergent is compatible with the high protein concentrated formulation.

Example 4:

The unexpected beneficial effect of acetic acid can be illustrated in the following experiment. Solutions of 161 mg/ml E25 in 0.1% (17.5 mM)acetic acid (pH 4.8) were dialyzed against i) 17.5 mM phosphate buffer with 145 mM NaCl (PBS) pH 7.4; ii) 17.5 mM acetate buffer pH 4.8; and iii) 17.5 mM citrate buffer pH 4.8. Unexpectedly, in the citrate buffer pH 4.8 solution E25 aggregated and the solution became white turbid. This did not happen in the other solutions. The phosphate buffer was more viscous than the acetate buffer solution. The phosphate buffer E25 solution became opalescent after one day at room temperature.

Example 5:

The viscosity of different E25 solutions was measured. All measurements were performed with a Paar Physica cone and plate rheometer at 23°C. The results are shown in Table 1 and Table 2 below.

Table 1:

E25 Samples	Viscosity η (mPa·s) at shear rate of γ=100 (1/s)	Viscosity η (mPa·s) at shear rate of γ= 225 (1/s)	Notes
97.4 mg/ml , 0.01% acetic acid	22.4	21.2	Beneficial effect of 0.01% acetic acid compared to water
99 mg/ml, in water	33.9	32.0	
222 mg/ml, 0.1% acetic acid	126	123	
222 mg/ml, 0.1% acetic acid, 50 mM CaCl2	66.6	63.2	
222 mg/ml, 0.1% acetic acid , 100 mM CaCl2	59.2	55	CaCl$_2$ decreases the viscosity
222 mg/ml, 0.1% acetic acid , 50 mM MgCl2	79.5	77.2	
222 mg/ml, 0.1% acetic acid , 100 mM MgCl2	67.9	64.5	MgCl$_2$ decreases the viscosity
222 mg/ml, 0.1% acetic acid ,50 mM NaCl	109	103	
222 mg/ml, 0.1% acetic acid ,100 mM NaCl	114	112	
222 mg/ml, 0.1% acetic acid ,150 mM NaCl	117	118	No effect of NaCl

Table 2:

E25 Samples	Viscosity η (mPa·s) at share rate of γ = 1	Notes
222 mg/ml, 0.1% acetic acid	368	
222 mg/ml, 0.1% acetic acid ,50 mM NaCl	351	
222 mg/ml, 0.1% acetic acid ,100 mM NaCl	1080	
222 mg/ml, 0.1% acetic acid ,150 mM NaCl	2140	NaCl increases the viscosity at very low shear rates

Example 6:

A solution of 161 mg/ml in 0.1% acetic acid was lyophilized in a glass vial.
After lyophilization the obtained E25 cake was difficult to solubilize with 0.1 % acetic acid.
However, the lyophilized E25 could be solubilized very quickly with a reconstitution solution

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of 0.1% acetic acid containing 100 mM CaCl$_2$. The lyophilized E25 was reconstituted at a concentration of 235 mg/ml. (the volume of the reconstitution solution was smaller than the initial volume of the solution). This example shows that CaCl$_2$ has unexpected beneficial effects in solubilizing E25 lyophilisates.

Example 7: General method for the preparation of high concentrated liquid
        formulations

The starting solution is a solution of purified antibody at low concentration (lower than the high concentrations of the invention) in an aqueous buffer, for example in the buffer resulting from the preceding process step (for example in the case of E25: 25 mM TRIS buffer pH 8 containing about 200 mM NaCl). The pH of this solution is adjusted to a value below the isoelectric point of the antibody, for example to pH 5, with an acid, for example with 5% acetic acid. The resulting solution is then concentrated and diafiltered by ultrafiltration, preferably in a tangential-flow filtration system, using a membrane able to retain quantitatively the antibody, for example with a cutoff of 30 kD or 10 kD.

In general the following 3-steps procedure applies:
• In a first step, the antibody solution is concentrated to an intermediate concentration, for example 40 mg/ml. Normally the retentate obtained is opalescent, due to antibody aggregation.
• In a second step, the concentrated solution is diafiltered with an aqueous acetic acid solution (for example 0.01% or 0.1% acetic acid) containing MgCl$_2$ or CaCl$_2$ (for example at concentration 50 mM or 100 mM) and optionally containing other additives (for example a sugar). The diafiltration is generally carried out at constant retentate volume, with at least 5 volumes, or preferably 8 volumes, of diafiltration buffer. During the diafiltration the antibody solution is turbid.
• In a third step, the diafiltered solution is further concentrated to a high concentration, for example higher or equal to 240 mg/ml. The final turbid retentate is then recovered out of the ultrafiltration system.
After an optional addition of additives (for example a detergent and eventually other excipients, e.g. sugars, buffering agents) and after filtration through a 0.2 μm filter, a high concentrated liquid formulation is obtained, which is clear and stable if stored at about 4°C.

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In a preferred embodiment of this general method, in order to process less turbid solutions, the following 5-steps procedure applies:

- In a first step, the antibody solution is concentrated to an intermediate concentration, for example 40 mg/ml. Normally the retentate obtained is opalescent, due to antibody aggregation.

- In a second step, the concentrated solution is diafiltered with an aqueous solution containing only acetic acid (for example 0.01% or 0.1% acetic acid). The diafiltration is generally carried out at constant retentate volume, with at least 5 volumes, or preferably 8 volumes, of diafiltration buffer. Normally, a decrease of the turbidity is observed during the diafiltration and the solution turns clear.

- In a third step, the diafiltered solution is further concentrated to a higher intermediate concentration, preferably of about 120 – 130 mg/ml. Then, a concentrated aqueous solution of $MgCl_2$ (or $CaCl_2$), for example at concentration 1 M, is added directly into the ultrafiltration system, to give approximately the desired resulting concentration (for example 50 mM or 100 mM). After mixing by retentate recirculation, a decrease of the retentate pressure is observed, due to the resulting lower viscosity. The retentate obtained remains clear or slightly turbid.

- In a fourth step, the solution is diafiltered with the same acetic acid solution as used for the first diafiltration (for example 0.01% or 0.1% acetic acid), but this time containing additionally $MgCl_2$ (or $CaCl_2$) at the desired concentration (for example 50 mM or 100 mM), in order to adjust exactly this concentration in the retentate. The diafiltration is generally carried out at constant retentate volume, with at least 5 volumes, or preferably 8 volumes, of diafiltration buffer.

- In a fifth step, the diafiltered solution is further concentrated to a high concentration, for example higher or equal to 240 mg/ml. The final clear or slightly turbid retentate is then recovered out of the ultrafiltration system.

After an optional addition of additives (for example a detergent and eventually other excipients, e.g. sugars, buffering agents) and after filtration through a 0.2 μm filter, a high concentrated liquid formulation is obtained, which is clear and stable if stored at about 4°C.

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Example 8: Preparation and viscosity of a formulation containing acetic acid and
           $MgCl_2$

About 12 ml of the liquid formulation [257 mg/ml E25, 0.1% acetic acid, 50 mM $MgCl_2$] were prepared by ultrafiltration in a tangential-flow filtration system (membrane area: 150 cm^2, membrane cutoff: 10 kD, hold up volume of the system: 9 ml, retentate pressure: 2 – 3 bar), according to the 5-steps procedure described in Example 7.

The starting solution was a solution of purified E25 antibody at concentration 4.8 mg/ml in a 25 mM TRIS buffer pH 8 containing about 200 mM NaCl. After pH adjustment to pH 5 with 5% acetic acid, the following steps were carried out:

- In a first step, the solution was concentrated to 40 mg/ml.
- In a second step, the concentrated solution was diafiltered at constant retentate volume with 8 volumes of 0.1% acetic acid.
- In a third step, the diafiltered solution was concentrated to 127 mg/ml and, after retentate recirculation during 5 minutes with the filtrate line closed, a sample was taken for viscosity measurement. Then, an aqueous solution of 1 M $MgCl_2$ was added directly into the ultrafiltration system, to give approximately a resulting $MgCl_2$ concentration of 50 mM. After reconcentration to the initial retentate volume (i.e. the volume before the addition of $MgCl_2$) and after retentate recirculation during 3 minutes with the filtrate line closed, a sample of the retentate was taken for viscosity measurement.
- In a fourth step, the solution was diafiltered at constant retentate volume with 8 volumes of 0.1% acetic acid containing 50 mM $MgCl_2$.
- In a fifth step, the diafiltered solution was concentrated to about 260 mg/ml. After recovery of the retentate out of the ultrafiltration system and filtration through a 0.2 μm filter, a sample was taken for viscosity measurement. An other sample was diluted to 200 mg/ml with 0.1% acetic acid containing 50 mM $MgCl_2$, also for viscosity measurement.

The viscosity measurements of the samples were performed with a Paar Physica cone and plate rheometer at 23°C and at a shear rate of 220 s^{-1}. The following results were obtained:

Process step:	E25 conc.	pH	Viscosity
in 0.1% acetic acid, before MgCl₂ addition:	127 mg/ml	4.43	12.1 mPa·s
in 0.1% acetic acid, after MgCl₂ addition:	127 mg/ml	4.48	8.27 mPa·s
in 0.1% acetic acid, 50 mM MgCl₂:	257 mg/ml	3.84	135 mPa·s
in 0.1% acetic acid, 50 mM MgCl₂:	200 mg/ml	3.82	37.1 mPa·s

Example 9: Viscosity of E25 formulations versus the acetic acid concentration

The same experiment as stated in Example 8 was carried out several times, changing only the acetic acid concentration used for the diafiltration buffers, but keeping the final MgCl₂ concentration equal to 50 mM. The different high concentrated E25 solutions obtained were then diluted to about 200 mg/ml, using the respective diafiltration buffers (i.e. the corresponding acetic acid solutions containing 50 mM MgCl₂), for pH and viscosity measurements.

The viscosity measurements were performed with a Paar Physica cone and plate rheometer at 23°C and at a shear rate of 220 s^{-1}. The following results were obtained:

Formulation buffer:	E25 conc.	pH	Viscosity
0.1 % acetic acid, 50 mM MgCl₂:	200 mg/ml	3.82	37.1 mPa·s
0.05 % acetic acid, 50 mM MgCl₂:	200 mg/ml	4.03	31.4 mPa·s
0.025 % acetic acid, 50 mM MgCl₂:	206 mg/ml	4.26	33.8 mPa·s
0.01 % acetic acid, 50 mM MgCl₂:	195 mg/ml	4.63	38.3 mPa·s
0.005 % acetic acid, 50 mM MgCl₂:	197 mg/ml	4.83	54.5 mPa·s
0.0025% acetic acid, 50 mM MgCl₂:	205 mg/ml	5.01	106 mPa·s
0.001 % acetic acid, 50 mM MgCl₂:	201 mg/ml	5.13	115 mPa·s
0 % acetic acid, 50 mM MgCl₂:	198 mg/ml	5.35	200 mPa·s

As shown by these results, when lowering the acetic acid concentration from 0.1% to 0% (at constant antibody concentration and at constant MgCl₂ concentration) the viscosity remains approximately constant in the concentration range between 0.1% and 0.01%, but increases drastically if the concentration is further reduced.

It was found that this „transition concentration" of about 0.0075% acetic acid corresponds to 1.3 mM, which corresponds to the E25 molar concentration corresponding to 200 mg/ml. Accordingly, in one embodiment of the invention the concentration of the acidic component of the invention is so chosen as to be about equal or above the molar concentration of the antibody of the aqueous solution or formulation of the invention.

Example 10: Viscosity of formulations containing acetic acid and either $MgCl_2$ or $CaCl_2$

About 18 ml of the liquid formulation [237 mg/ml E25, 0.01% acetic acid, 50 mM $MgCl_2$] were prepared by ultrafiltration in a tangential-flow filtration system (membrane area: 150 cm², membrane cutoff: 10 kD, hold up volume of the system: 10 ml, retentate pressure: 2.5 – 4 bar), according to the 3-steps procedure described in Example 7:

The starting solution was a solution of purified E25 antibody at concentration 4.8 mg/ml in a 25 mM TRIS buffer pH 8 containing 200 mM NaCl. After pH adjustment to pH 5 with 5% acetic acid, the following steps were carried out:

- In a first step, the solution was concentrated to 40 mg/ml.
- In a second step, the concentrated solution was diafiltered at constant retentate volume with 8 volumes of 0.01% acetic acid containing 50 mM $MgCl_2$.
- In a third step, the diafiltered solution was concentrated to 230 – 240 mg/ml. After recovery of the retentate out of the ultrafiltration system and filtration through a 0.2 µm filter, two samples was taken for viscosity measurement (the first one as is, the second one after addition of 0.02% of Tween 20). Two other samples were diluted to about 210 mg/ml with 0.01% acetic acid containing 50 mM $MgCl_2$, also for viscosity measurement (the first one as is, the second one after addition of 0.02% of Tween 20).

The same experiment was repeated, but using $CaCl_2$ instead of $MgCl_2$, giving the liquid formulation [233 mg/ml E25, 0.01% acetic acid, 50 mM $CaCl_2$].

The viscosity measurements were performed with a Paar Physica cone and plate rheometer at 23°C and at a shear rate of 220 s^{-1}. The following results were obtained:

Formulation buffer:	E25 conc.	Viscosity
0.01% acetic acid, 50 mM MgCl₂:	237 mg/ml	83.5 mPa·s
0.01% acetic acid, 50 mM MgCl₂, 0.02% Tween 20:	237 mg/ml	86.6 mPa·s
0.01% acetic acid, 50 mM CaCl₂:	233 mg/ml	60.5 mPa·s
0.01% acetic acid, 50 mM CaCl₂, 0.02% Tween 20:	233 mg/ml	59.1 mPa·s
0.01% acetic acid, 50 mM MgCl₂:	211 mg/ml	40.5 mPa·s
0.01% acetic acid, 50 mM MgCl₂, 0.02% Tween 20:	211 mg/ml	42.1 mPa·s
0.01% acetic acid, 50 mM CaCl₂:	207 mg/ml	34.8 mPa·s
0.01% acetic acid, 50 mM CaCl₂, 0.02% Tween 20:	207 mg/ml	31.6 mPa·s

As shown by these results, the viscosity values are slightly lower if $CaCl_2$ is used instead of $MgCl_2$. Moreover, the Tween 20 at concentration 0.02 % has no influence on the viscosity.

Example 11: Preparation and stability of high concentrated liquid formulations

The three following high concentrated liquid formulations were prepared by ultrafiltration (about 65 ml each, starting with E25 drug substance without Tween), according to the 5-steps procedure described in Example 7:

Formulation #	F1	F2	F3
Lot #	NVP-IGE025-01PP01	NVP-IGE025-01PP02	NVP-IGE025-01PP03
Composition:			
E25	196 mg/ml	201 mg/ml	167 mg/ml
acetic acid	0.1 %	0.1 %	0.05 %
MgCl₂	50 mM	50 mM	50 mM
Mg-acetate	---	30 mM	45 mM
Trehalose	27 mg/ml	---	---
Tween 20	0.02 %	0.02 %	0.02 %
pH	4.50	4.95	5.20
Tonicity	273 mOsm/kg	252 mOsm/kg	277 mOsm/kg
Viscosity (at 220 s⁻¹; 23°C)	39.9 mPa·s	48.3 mPa·s	19.5 mPa·s

These formulations were put on a stability program and were found to be stable after 6-months storage at 5°C (study ongoing). The following assays were carried out: SEC (size-exclusion chromatography), HIC (hydrophobic-interaction chromatography after papain-digestion) and Bioassay (IgE-Receptor binding inhibition assay):

Formulation #	F1	F2	F3
SEC:	% Monomer:	% Monomer:	% Monomer:
start	99.1	98.9	99.1
1 month (5°C)	98.5	98.6	98.6
3 months (5°C)	99.1	98.9	99.0
6 months (5°C)	98.7	98.3	98.7
HIC:	% Unmodified:	% Unmodified:	% Unmodified:
start	63	62	58
1 month (5°C)	63	62	63
3 months (5°C)	60	60	60
6 months (5°C)	59	61	62
Bioassay:	% Specific Activity:	% Specific Activity:	% Specific Activity:
start	105	107	100
1 month (5°C)	75	79	79
3 months (5°C)	97	95	99
6 months (5°C)	111	99	85

As shown by these results, the three liquid formulations have a stability of at least 6 months at 5°C.

Example 12: Viscosity of aqueous solutions of E25 at high concentration containing only acetic acid at low concentration

About 31 ml of the aqueous solution [127 mg/ml E25, 0.1% acetic acid] were prepared by ultrafiltration in a tangential-flow filtration system (membrane area: 150 cm^2, membrane cutoff: 10 kD, hold up volume of the system: 9 ml, retentate pressure: 2 – 3 bar), according to the three first steps of the 5-steps procedure described in Example 7:

The starting solution was a solution of purified E25 antibody at concentration 4.8 mg/ml in a 25 mM TRIS buffer pH 8 containing about 200 mM NaCl. After pH adjustment to pH 5 with 5% acetic acid, the following steps were carried out:

• In a first step, the solution was concentrated to 40 mg/ml.

- In a second step, the concentrated solution was diafiltered at constant retentate volume with 8 volumes of 0.1% acetic acid.
- In a third step, the diafiltered solution was concentrated to about 120 mg/ml and a sample was taken for viscosity measurement.

The same experiment was carried out several times, changing only the acetic acid concentration used for the diafiltration.

The viscosity measurements were performed with a Paar Physica cone and plate rheometer at 23°C and at a shear rate of 220 s^{-1}. The following results were obtained:

Acetic acid concentration	E25 conc.	Viscosity
0.1 % (i.e. 17.3 mM)	127 mg/ml	12.1 mPa·s
0.1 % (i.e. 17.3 mM)	111 mg/ml	7.4 mPa·s
0.05 % (i.e. 8.7 mM)	118 mg/ml	9.4 mPa·s
0.025 % (i.e. 4.3 mM)	121 mg/ml	13.8 mPa·s
0.01 % (i.e. 1.7 mM)	121 mg/ml	17.8 mPa·s
0.005 % (i.e. 0.87 mM)	120 mg/ml	24.4 mPa·s
0.0025 % (i.e. 0.43 mM)	115 mg/ml	25.4 mPa·s
0.001 % (i.e. 0.17 mM)	120 mg/ml	26.7 mPa·s
0 % (i.e. water alone)	116 mg/ml	47.2 mPa·s

As shown by these results, the beneficial effect of acetic acid compared to water is already observed at an acetic concentration as low as 0.17 mM, which allows to prepare an antibody solution at a concentration of 120 mg/ml having a viscosity significantly lower than 50 mPa·s (i.e. the corresponding viscosity obtained with water alone).

Example 13: Viscosity of aqueous solutions of E25 containing only 0.1% acetic acid, in function of the antibody concentration

The same experiment as stated in Example 12 was repeated using 0.1% acetic acid for the diafiltration step, but this time the diafiltered solution was concentrated to about 240 mg/ml (instead of 120 mg/ml). After recovery of the retentate out of the ultrafiltration system and filtration through a 0.2 μm filter, a sample was taken for viscosity measurement. Other

samples were taken as well, for viscosity measurements after various dilution steps with 0.1% acetic acid.

The viscosity measurements were performed with a Paar Physica cone and plate rheometer at 23°C and at a shear rate of 220 s^{-1}. The following results were obtained:

Acetic acid conc.	E25 conc.	Viscosity
0.1 %	240 mg/ml	225 mPa·s
0.1 %	220 mg/ml	125 mPa·s
0.1 %	200 mg/ml	63 mPa·s
0.1 %	180 mg/ml	40 mPa·s
0.1 %	170 mg/ml	35 mPa·s
0.1 %	148 mg/ml	20 mPa·s
0.1 %	127 mg/ml	12 mPa·s
0.1 %	85 mg/ml	6 mPa·s

As shown by these results, the beneficial effect of acetic acid allows to prepare antibody solutions at a concentration up to about 180 mg/ml, having a viscosity significantly lower than 50 mPa·s.

Example 14: Use of citric acid as acidic component

About 19 ml of an aqueous solution of E25 at a concentration of about 155 mg/ml in purified water having a pH of 4.4 – 4.6 adjusted with citric acid were prepared by ultrafiltration in a tangential-flow filtration system (membrane area: 150 cm^2, membrane cutoff: 10 kD, hold up volume of the system: 10 ml, retentate pressure: 2 – 3 bar), according to a procedure similar to the 3-steps procedure described in Example 7:

The starting solution was a solution of purified E25 antibody at concentration 4.8 mg/ml in a 25 mM TRIS buffer pH 8 containing about 200 mM NaCl. After pH adjustment to pH 4.7 with 0.5 M citric acid, corresponding to a resulting citric acid concentration of about 6.6 mM, the following steps were carried out:

- In a first step, it was tried to concentrate the solution to 40 mg/ml. But the filtrate flow decreased immediately very quickly, so that it was not possible to carry out this concentration step at the pH value of 4.7. In order to recover a normal filtrate flow, the pH of the solution was lowered by stepwise addition of small amounts of 0.5 M citric acid. Neither pH 4.4 nor pH 4.2 was low enough to allow a satisfactory filtrate flow. Finally, the concentration step was possible only after pH lowering to pH 4.0, corresponding to a resulting citric acid concentration of about 9 mM.

- In a second step, the concentrated solution was diafiltered at constant retentate volume with 8 volumes of purified water having a pH of about 4.4, preliminarily adjusted with a few droplets of 0.5 M citric acid, corresponding to a resulting citric acid concentration in the range of about 0.05 to 0.1 mM.

- In a third step, the diafiltered solution was concentrated as high as possible. After recovery of the retentate out of the ultrafiltration system and filtration through a 0.2 µm filter, a sample was taken for concentration and pH measurements.

The maximal reachable concentration was 155 mg/ml, with a resulting pH of 4.5.
In comparison, the maximal concentration obtained by using 0.1 % acetic acid (without other additives) with the same ultrafiltration equipment was about 240 mg/ml.

Moreover, a sample of this concentrated solution (155 mg/ml, pH 4.5) was taken for addition of sodium citrate buffer of pH 4.5 to a foreseen final buffer concentration of 17.5 mM. But already after the addition of the first droplets (of 1M sodium citrate buffer pH 4.5), E25 aggregated immediately and the solution became white turbid, turning soon into a white solid gel. If 1M $MgCl_2$ (instead of 1M sodium citrate pH 4.5) is added to the final concentrated solution of Example 14 (to a final $MgCl_2$ concentration of 50 mM), the solution remains clear.

Claims:

1. A stable aqueous solution comprising an antibody at a concentration of at least 50 mg/ml, and further comprising at least one acidic component.

2. The aqueous solution of claim 1 consisting of, or consisting essentially of, an antibody at a concentration of at least 50 mg/ml and an acidic component.

3. The aqueous solution of claim 1 or 2, wherein the acidic component is present in a final concentration of at least 0,001%, preferably at least 0,01%.

4. The aqueous solution of any of claims 1 to 3 wherein the acidic component is acetic acid.

5. The aqueous solution of any of claims 1 to 4 wherein the pH of said aqueous solution is above pH3, preferably between pH 3 and pH 6.

6. The aqueous solution of any of claims 1 to 5 wherein the antibody has an isoelectric point between pH 6 and pH 8.

7. The aqueous solution of any of claims 1 to 6 wherein the antibody is selected from anti-IgE antibodies E25, E26, E27 or their biologically active fragments or derivatives.

8. The aqueous solution of any of claims 1 to 7 having a stability of at least 1 year at about 4°C.

9. The aqueous solution of any of claims 1 to 8, further including $CaCl_2$.

10. The aqueous solution of any of claims 1 to 9, further including $MgCl_2$.

11. The aqueous solution of any of claims 1 to 10, further including at least one additive.

12. The aqueous solution of claim 11, wherein the additive is Tween 20.

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13. The aqueous solution of claim 11, wherein the additive is a sugar.

14. The aqueous solution of claim 13, wherein the sugar is selected from the group of trehalose, sucrose, mannitol, sorbitol, fructose, maltose, lactose or a dextran.

15. The aqueous solution of claim 11, wherein the additive is a buffering agent.

16. The aqueous solution of any of claims 1 to 15 wherein said aqueous solution is isotonic.

17. A nasal spray comprising the aqueous solution as claimed in any preceding claim.

18. A slow release formulation comprising the aqueous solution as claimed in any preceding claim.

19. The slow release formulation of claim 18 selected from the group of polymeric nano- or microparticles, or from gels.

20. The slow release formulation of claim 19, wherein the gel is a hylauronic acid gel.

21. A delivery system which contains the aqueous solution as claimed in any preceding claim, selected from the group of single use injection syringes or inhalation devices.

22. Use of the aqueous solution according to any of claims 1 to 16 for the production of a delivery system for the treatment of a disease.

23. Use of an acidic component for the preparation of an aqueous solution comprising an antibody having a concentration of at least 50 mg/ml.

24. Use of the aqueous solution according to any of claims 1 to 16 in a drying or freeze- drying process.

25. A process for the preparation of a aqueous solution according to any of claims 1 to 16, which process comprises admixing an antibody with an acidic component.

26. A process for the preparation of a therapeutical liquid formulation comprising an antibody, wherein in a first step an aqueous solution including an antibody at a concentration of at least 50 mg/ml and at least one acidic component is prepared, and, in a second step, at least one pharmaceutically acceptable additive is added to said aqueous solution.

27. A process for the preparation of a therapeutical liquid formulation comprising an antibody at a concentration of more than 50 mg/ml, wherein
   - in a first step an antibody solution in a suitable buffer is concentrated to a concentration of between about 10 mg/ml and about 50 mg/ml;
   - in a second step, the concentrated solution obtained in the first step is diafiltered with an aqueous solution of at least one acidic component, optionally containing $MgCl_2$ and/or $CaCl_2$ and/or further suitable additives; and,
   - in a third step, the solution obtained in the second step is further concentrated to a concentration of more than 50 mg/ml.

28. A process for the preparation of a therapeutical liquid formulation comprising an antibody at a concentration of more than 50 mg/ml, wherein
   - in a first step an antibody solution in a suitable buffer is concentrated to a concentration of between about 10 mg/ml and about 50 mg/ml;
   - in a second step, the concentrated solution obtained in the first step is diafiltered with an aqueous solution of at least one acidic component;
   - in a third step, the solution obtained in the second step is further concentrated to an intermediate concentration of between about 100 and about 200 mg/ml, preferably between about 100 and about 150 mg/ml;
   - in a fourth step, the intermediate concentrated solution obtained in the third step is diafiltered with an aqueous solution of at least one acidic component and further containing $MgCl_2$ and/or $CaCl_2$ and/or further suitable additives; and,
   - in a fifth step, the solution obtained in the fourth step is further concentrated to a concentration of more than 150 mg/ml.

29. The process of claim 28, wherein between the third and fourth step a solution of
    $MgCl_2$ and/or $CaCl_2$ and/or further suitable additives is directly added to the
    intermediate concentrated solution obtained in the third step.

30. The process of claim 25 to 29, wherein the acidic component is present in a final
    concentration of at least 0,001%, preferably at least 0,01%.

31. The process of claim 25 to 30, wherein the acidic component is acetic acid.

32. The process of claim 25 to 31, wherein the pH of said aqueous solution is above
    pH3, preferably between pH 3 and pH 6.

33. The process of claim 25 to 32, wherein the antibody has an isoelectric point between
    pH 6 and pH 8.

34. The process of claim 25 to 33, wherein the antibody is selected from anti-IgE
    antibodies E25, E26, E27 or their biologically active fragments or derivatives.

35. A therapeutical liquid formulation obtained by a process of any of claims 26 to 34.

36. A therapeutical liquid formulation obtainable by a process of any of claims 26 to 34.

37. The therapeutical liquid formulation of claims 35 or 36 having a stability of at least 1
    year at about 4°C.

38. The therapeutical liquid formulation of claims 35 to 37, further including $CaCl_2$ at a
    concentration of between 50mM and 200mM.

39. The therapeutical liquid formulation of claims 35 to 38, further including $MgCl_2$ at a
    concentration of between 50mM and 200mM.

40. The therapeutical liquid formulation of claims 35 to 39, further including at least one
    additive.

41. The therapeutical liquid formulation of claim 40, wherein the additive is Tween 20.

42. The therapeutical liquid formulation of claim 40, wherein the additive is a sugar.

43. The therapeutical liquid formulation of claim 42, wherein the sugar is selected from the group of trehalose, sucrose, mannitol, sorbitol, fructose, maltose, lactose or a dextran.

44. The therapeutical liquid formulation of claim 40, wherein the additive is a buffering agent.

45. The therapeutical liquid formulation of any of claims 35 to 44, wherein said liquid formulation is isotonic.

46. A process for the preparation of a therapeutical liquid formulation comprising an antibody, which process comprises adding an acidic component on the last purification step of the preparation of said antibody.

47. The aqueous solution according to any of claims 1 to 16 for use in medicine.

48. Use of an aqueous solution according to any of claims 1 to 16 for the manufacture of a medicament for the treatment of disease.

49. Use of an aqueous solution according to any of claims 1 to 16 for the manufacture of a medicament for the treatment of an allergic disease.